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## PURPOSES IN MEDICAL RESEARCH

### AN INTRODUCTION TO THE JOURNAL OF CLINICAL INVESTIGATION

By ALFRED E. COHN

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Custom has varied in the history of medical journalism, certain journals were introduced to their readers without explicit statements by their editors of the purposes which the new publications were to serve. In these instances it was left to chance or to the general knowledge of the contemporary public to find within its pages a justification for the new venture. Other journals have been explicit in the avowal of their objects. Both methods have advantages, both have disadvantages. In a discipline as old as medicine, which has continuously engaged the profound interest of men for as many centuries as has any of the other subjects in which men have exercised curiosity and the desire for knowledge, it is fitting in the interests of definiteness and with the view of making an exact statement of our conceptions, as well as in attempting to anticipate the natural inquiry of our contemporaries, to define the motives which suggest this new publication.

There is a pitfall here, which should be avoided. In the attempt to explain the purposes which actuate the publication of a new journal, the impulse may be, as Naunyn<sup>1</sup> pointed out in the case of Wunderlich, to make too precise the limits within which the thought which underlies the undertaking is to be confined. The doors in medicine must naturally be kept open so that influences, no matter whence derived, may contribute their share to the understanding and elucidation of the problems which constitute the proper province of medicine. But that a danger lies here history has made amply apparent. For there has never been a time either in the ancient or in the modern world when medicine was far removed from the influences of neighbor-

<sup>1</sup>Naunyn B., *Deut. Arch. f. Klin. Med.* 1922, cxi, 1-27. *Die deutsche Heilkunde vom Anfang des neunzehnten Jahrhundert*

ing disciplines It has in point of fact often benefited by importing for its own guidance the conceptions which prevailed in other domains of inquiry, whether these conceptions were borrowed from the physical or from the biological world But it has also suffered from this habit The latest bondage into which medicine was led and from which it was freed less than a century ago was due to the influence of romantic metaphysics at the beginning of the 19th century The record of the history of medical progress gives us no assurance that, without constant watchfulness, we shall escape in the future enticements from the proper direction which thought and activity might pursue in the study of human disease

Since the renaissance, men of science have indeed been continuously eager to escape from those influences which tended to focus their interests on the contemplation alone of natural phenomena and have sought, under the stimulus supplied by Francis Bacon, to enlarge knowledge by coming actually into contact with the facts and forces of nature But they have likewise been alive to the dangers inherent in this pursuit, for side by side with the collection of facts and the making of experiments, rules were sought by the application of which science might in some measure be assured that in the management of its discoveries it was proceeding along paths which led to correct generalization That is to say, the method of deduction in natural science as the sole method of investigation was finally abandoned and the method of induction, of experiment, was added It was soon found that even this reform did not suffice, infinite experimentation might very well produce facts in endless variety But facts, divorced from meaning have never for long periods of time held the attention of men Science has constantly insisted on arranging facts in order, with the view to arriving at some statement of their significance How soon after the time of Bacon this problem came prominently into the view of experimental scientists the following observations of Boyle<sup>2</sup> show

if men could be perswaded to mind more the Advancement of Natural Philosophy than that of their own reputations, 'twere not me-thinks very uneasie to make them sensible, that one of the considerablest services that they

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<sup>2</sup> Boyle, R , Certain Physiological Essays, London, 1661, 8-9

could do Mankind were to set themselves diligently and industriously to make Experiments and collect Observations, without being over-forward to establish Principles and Axioms, believing it uneasy to erect such Theories as are capable to explicate all the Phaenomena of Nature, before they have been able to take notice of the tenth part of those Phaenomena that are to be explicated Not that I at all disallow the use of Reasoning upon Experiments, or the endeavouring to discern as early as we can the Confederations, and Differences, and Tendencies of things For such an absolute suspension of the exercise of Reasoning were exceeding troublesome, if not impossible so in Physiology it is sometimes conducive to the discovery of truth to permit the Understanding to make an Hypothesis in order to the Explication of this or that Difficulty, that by examining how farre the Phaenomena are, or are not, capable of being salv'd by that Hypothesis, the Understanding may ev'n by its own Errors be instructed For it has been truly observed by a great Philosopher, That Truth does more easily emerge out of Error than Confusion That then that I wish for, as to Systems, is this, That men in the first place would forbear to establish any Theory, till they have consulted with (though not a fully competent Number of Experiments, such as may afford them all the Phaenomena to be explicated by that Theory, yet) a considerable number of Experiments in proportion to the comprehensiveness of the Theory to be erected on them And in the next place, I would have such kind of superstructures look'd upon only as temporary ones, which though they may be preferr'd before any others, as being the least imperfect, or, if you please, the best in their kind that we yet have, yet are they not entirely to be acquiesced in, as absolutely perfect, or uncapable of improving Alterations

Medicine has shared this interest in arrangement with the rest of science The significance of Sydenham is to be found precisely in this connection But how inadequate arrangement is in itself in the attempt to arrive at significance is to be observed in the further extension of his method by the later systematists, Sauvage and Linné

Activity in certain other directions has likewise resulted in disappointing experience This result is seen, for instance, in the application to medicine of methods developed in other fields of inquiry Illustrations of the futility of this sort of activity are to be found in the work of Borelli and the iatro-mathematical school, in that of van Helmont and the iatro-chemists, and in that of Boerhaave, Cullen and others in attempts to introduce isolated methods of measuring, as for instance of temperature, into the study of disease In the first instance, methods were used which perhaps could not lead to an understanding of morbid phenomena, in the second, the methods were in point of fact not developed sufficiently to render profitable their

application to disease processes. Methods of induction if used alone failed, when they were merely borrowed, just as had the method of deduction. Medicine was not alone in these experiences. Indeed, if we are to credit reporters of the history of other sciences, similar experiences have been encountered in them so that it is now a general conclusion that, in order to achieve development in natural science, both methods should be employed.

It is a noteworthy observation that just at the time when Borelli, van Helmont, and others were seeking to advance medicine by importing into it the developments made in other disciplines and were meeting with what proved to be indifferent success, parallel developments were taking place due to the work of men whose interest originated in speculation aroused primarily by curiosity as to the behavior of the body itself. Out of this curiosity came the genuine advances of Mayow, Harvey and Sydenham. But the employment of a method presupposes that in a proposed inquiry the use of the method selected is advantageous in the solution of the problem. It has just been pointed out that medicine has before now been urged to adopt methods believed either by others or by medical men themselves to be advantageous in medical research. Error lay at the basis of this belief. That was true of the nature philosophers in Germany, with the result that for a generation medicine became a branch of metaphysics. That was true of the mechanics and mathematics of the 17th century when applied to medicine under the influence of Borelli, it was true of chemistry "applied" to medicine by van Helmont. The same erroneous program was proposed by Johannes Muller when he and his successors urged the "application" of physiology to medicine. Mathematics, mechanics, physics, chemistry, physiology as independent disciplines has each had its proper objects of inquiry, all have been aware of their appropriate problems in the phenomenal world. Their signal achievements are the common knowledge and have been the wonder of all men. Nor can there be doubt that the interest which their pursuit has aroused has exerted profound influence on medicine itself. But the primary objects of interest in medicine cannot properly be stated in terms appropriate to them.

Medicine must, like the other sciences, be properly credited with having specific objects of interest on its own account. If it is true that

medicine has not always been clear as to what these objects are, this may be due to the fact that the definition of its objects has not always been clear. It may perhaps be for this reason that it has so often been deflected from the straight path of its proper pursuit. For it cannot be the object of medicine or of any other discipline to "apply" the methods of other sciences to itself, whether of anatomy or physiology, whether of physics or chemistry. Medicine in the light of its history might properly pause at each new stage of its development and make the attempt to define for itself its legitimate scope and objects. It might do what Sir Philip Sidney said he had done in deciding how he had best write

Fooll! said my muse to me, look in thy heart, and write

If it attempts to do so now it will not be the first time that medicine has followed the advice of Sidney. We have, as all those interested in the progress of medicine know, for some time been inquiring whether medicine is entitled to be called a science. To us the answer to this question is clear and unequivocal. It is clear because of the nature of the case. The phenomena of interest in medicine are the phenomena of disease as these are manifest in affected persons. They are phenomena which exist as concrete entities in nature, they are indivisible, and they fall within the province of no other inquiry. They constitute the proper concern of medicine. Nor are the phenomena of disease the combination or resultants merely of other forces. They are not the resultants of forces known in physics and chemistry, nor in physiology and mathematics, nor the resultants of any combination of these. Rapid and shallow breathing for instance, as an appearance familiar in disease, may depend on a derangement of the familiar Hering-Breuer reflex, or it may depend on anoxaemia, or it may depend on a high hydrogen ion concentration. But irrespective of how this type of breathing is conditioned, it remains a unique phenomenon, even though the terms in which it is characterized are anatomical or physiological or chemical. Anatomy or physiology or chemistry may supply the methodology for analyzing the occurrence, but the occurrence is something apart from and over and above the factors into which it can be resolved. Heart failure presents the opportunity of another illustration. Phenomena are the basis



of a science, not the techniques by which phenomena are elucidated. Those of disease are, as has been said, indivisible phenomena, as indivisible as are those of botany or zoology or paleontology. When we come to the question of how to investigate them we find that they are to be studied by no single methodology any more than are those of the sciences just mentioned. The methods to be employed are those which are appropriate to illuminating the specific problems in question. In paleontology, the methods may be those of geology or comparative anatomy or petrology, in biology, they may be those of physiology or chemistry or physics.

"The aim of medicine," says Laennec,<sup>3</sup> "is the cure of disease." And he added that there were a multitude of ways by which this end might be attained. He singled out three especially for mention, that of the empiricists, who considered it sufficient to distinguish diseases by their apparent signs, second, that of those who believed it possible to disclose the causes of disease without giving themselves the trouble of learning their effects, and third, that of those who believed it was necessary to understand the diseases. We should perhaps add by way of interpreting or perhaps of supplementing Laennec's meaning, that we believe it necessary as the basis of therapeutics to understand the mechanisms, that is to say, the processes which underlie the manifestations of disease, for it is these which it is one of our functions to attempt to correct. That is our practical aim. We have learned a lesson also in another direction. It is that, as in other disciplines, learning may be pursued for its own sake. And the reason for this is two-fold. Men have learned that the direct is not always the shortest road to the attainment of their objects. It is true that results ultimately of practical value have issued from disinterested learning. But this argument still is based on utility and leaves many persons imbued with natural curiosity without enthusiasm. It is perhaps not unfair to say that these disinterested students have not been made welcome in medicine as they have been in other departments of learning. And this is a defect in our organization even if it represents no defect in our conceptions. The problems of disease offer legitimate objects of inquiry as do problems in physiology and may be pursued

<sup>3</sup> Laennec, R. T. H., Archives Generales de Medicine, 1823 1, 5

in the same spirit The illumination which has resulted from study of this kind requires no defence Its value in the development of science is sufficiently established

Medicine has not always given so frank an answer as to its function as the answer of Laennec, on occasion being over modest, on occasion being overwhelmed by the meagreness of its own success in comparison with that of other sciences, on other occasions still, being imperfectly aware of its purpose If we adopt the aim of medicine as Laennec stated it we may still fail to agree, as he intimates, on how this object is to be attained On certain preliminary matters, however, we cannot fail to agree First, we must continue to classify diseases Second, we must pursue our studies by the methods common to the natural sciences For having drawn our attention afresh to the underlying importance to be attached to the procedure of classification proposed by Sydenham we are indebted to Professor Faber of Copenhagen No one now interested in disease, would willingly dispense with the aid which has been gained by the identification and grouping of diseases It is necessary only to refer to the fevers to see how by their grouping, knowledge or perhaps better understanding of them has been gained The need for continuing this activity is still present Facts and relations are continuously being discovered, new categories are still being suggested The arrangement and classification of these often precedes the development of adequate knowledge for their comprehension. The significance of nosology for hygiene is immediately apparent Hygiene has quickly learned its lesson It has learned, where it has been successful, that the control of disease depends on preventing the entrance into the body of disease producing agents by ingestion or by controlling the habits of intermediate hosts Further than this the classification of fevers occasioned the first great success of nosology in the domain of therapeutics Certain directions in which effort may be expended have now been clearly indicated But the success of nosology in other groups of disease has not yet been clearly displayed Other categories have indeed been separated, depending on disturbances either of the organs or of systems and relations within the body And, finally, we have come to recognize still others which depend on heredity or on constitutional organization It is necessary only to mention psychic disturbances and in-

sanity to appreciate the fact that these, since the time when they were admitted to be diseases, have been treated as constituting a separate group

Viewed from a different angle it has been customary to regard diseases as falling into groups depending on their duration, on their being either acute or chronic. A relation to the possibility of recovery has usually been implied, but is not yet clearly defined. The acute diseases, it is scarcely necessary to say, include the fevers, the communicable diseases, and although usually not brief in duration, tuberculosis, syphilis and rheumatic fever may be grouped here. In this group the etiological agents are either already known or the belief is entertained that their discovery is possible and awaits the use of suitable methods for the purpose. Indeed the success in treatment which has already attended the discovery of the etiological agents in this group has been the basis for the belief that the discovery of their etiology is the key to the solution of the therapeutics of disease in general. In this expectation it is possible even now to see an error unless the consequences of an injury can be prevented either at the time of onset or in an early stage after the injury is received. So happy an outcome may, however, not always be possible in states which involve constitutional manifestation, or faulty "anlage" and the infirmities of old age. For some of these, medicine must still be under the necessity of providing relief, for others, such as exophthalmic goitre, of the means of providing correction, and of others still, such as diabetes, of preventing its occurrence. In the case of the so-called chronic diseases, in Bright's disease, heart disease, and chronic degenerative diseases of other organs we may perhaps look forward to the time when, should they prove to be preventable, a technique for accomplishing this result becomes available. But that time is not yet. Therapeutics must perforce concern itself therefore with the later states in the abnormal conditions which succeed those attending the infliction of injury and the early stages when the arrest of its operation may still be possible. It is unnecessary to recall the fact that a difficulty arises in the circumstance that the early stage is often not to be detected. An instance of this difficulty is seen in the establishment of mitral stenosis, the detection of which may be delayed for several years after an infection so slight as scarcely to have aroused anxiety, or of Bright's

disease, many years after the patient has passed through an attack of scarlet fever so mild as to have been detected only by the occurrence of a contact infection. In the later stages, when they can be detected, the therapeutics of diseases of this nature, that is to say of the heart and the kidneys, are no longer to be managed on the basis of etiology. These diseases constitute new states, the management of which must be undertaken with the new set of circumstances in view. So far etiological classification has been an approach to therapeutics in the group of communicable diseases only, where control is associated with prevention, or with the destruction of the causative agent in the host, the problem is that of the control of a foreign substance introduced into the body, the damage from which is combatted either with or without stimulation of the physiological reserves which the body can provide. The problem is the problem of invasion, it differs from the problem of continuous adjustment and control which derangements of the functions of organs and of bodily systems necessitate.

Accurate classification and a knowledge of the processes of disease and their relief and cure are then the proper objects of inquiry in medicine. No other discipline is, as has been said, primarily concerned with disease, nor has it contact with patients who exhibit the manifestations of disease. On account perhaps of the social importance of epidemic diseases, bacteriologists as apart from physicians have it is true busied themselves with the communicable affections. But the diseases due to microbic agents have after all a curious external relation not common to other disease groups, their prevention, their management, as hygiene prevents and manages them, requires no necessary contact with infected individuals. Management so far as cure is concerned naturally involves an equipment different from that of the bacteriologist. It is, however, remarkable that development in this direction, perhaps with the exception of lobar pneumonia, has not been due to the efforts of those concerned with the care of patients. It has been especially true of the identification of bacterial agents of disease, that medicine is indebted for this advance to bacteriologists, not to its own practitioners or professors. A dependence on the outside world for the solution of its problems is in part a reproach to medicine. Probably no injury has yet been suffered by society as a result of this dependence. But those advances

that depend on knowledge of disease in patients and on actual direct contact with diseased persons, have been made by the practitioners of medicine themselves. In this way is to be explained the significance Sydenham, Jenner and Laennec have for us, they have taught us the use of the classification of diseases, the fact that fevers are preventable, an approach to the diagnosis of visceral disease by means not immediately obvious. These have after all constituted the primary advances in medicine. It is this experience which encourages us in the belief that the development of medicine is in all probability the work of physicians properly trained and supplied with adequate equipment.

If this is the teaching of history, and these the problems of medicine, the future pathway becomes clear. We are concerned with the therapeutics both of acute and of chronic diseases as well as with the health of the body and the relief from its disabilities during the years of its decline. The development of the therapeutics of infectious diseases dependent on the discovery of bacteria has for two generations been so absorbing as to dwarf the interest medicine has always displayed in conditions associated with derangement of the organs and with the ailments of advancing age. But the latter rather than the former group counts the greater number of victims—a number which is the greater, the greater is the success which is achieved in the solution of problems connected with immunity and hygiene. The diseases of the later years occasion what one might name the therapeutics of physiological disharmony. They present conditions which give rise to extreme difficulty in therapeutics. Their nature is still obscure, in large measure because the mechanisms on which they depend have scarcely been analysed. Success in treating them can scarcely be expected until more knowledge has been accumulated of the normal mechanisms on a deviation from which they depend.

Medicine has, therefore, significant tasks, tasks of great complexity. That we are aware of them is evident when the efforts are reviewed which are being made in the contemporary study of Bright's disease, in the study of heart failure, in the study of hormonal derangements. We have begun to study these diseases which involve abnormal physiological processes as scientists always study, by whatever means natural science has to offer which promise success. We are engaged

now in the struggle to fit ourselves for the work of overcoming the difficulties involved in mastering the methodology we must use—be it physics, physiology, nosology or chemistry. These are, it need scarcely be pointed out, the methods employed likewise in biology, that the methods are the same is not surprising, in view of the fact that the living system which is studied in medicine does not differ from that which is the concern of biology in general, except that one has in medicine not always the advantage of adopting simple material to serve the purposes of one's experiments.

This, then, is the task which academic medicine in the United States, now become self-conscious, has set itself, it is the task of Clinical Investigation. Its business involves a legitimate interest in learning as well as a means for furthering the methods which lead to the cure of disease. It is vitally concerned in the success of both these projects. It ought, as it has been, to be concerned with the arrangements, both in education and in organization for accomplishing its ends. We must appreciate the fact that there is perhaps no single road of salvation open, the search for the single road has often led hunters far afield.

To give substance to ideas like these is the purpose which lies behind the work of the new university clinics which are being founded in many parts of our country. They mean to take on new functions. Those on which they lay emphasis, indicate the adoption of a wider interest in the problems of concern to medicine. In addition to the traditional responsibility for teaching they avow the desire to contribute to an increase of knowledge. They are drawing to themselves new men, trained in a new way, they are being supplied with new hospitals properly equipped with laboratories in which to pursue what Bernard called the *observation provoquée*. These activities testify to the development of a new spirit. This is the spirit which has called the American Society for Clinical Investigation into being. It is the spirit to which the Journal for Clinical Investigation desires to give expression. It is a spirit which the Journal wishes to foster and of which it hopes to be worthy.



# STUDIES ON THE SPECIFIC GRAVITY OF THE URINE<sup>1</sup>

By O H PERRY PEPPER

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(Received for publication, May 19, 1924)

In a study of the effect of the various solids of the urine upon the specific gravity of the urine, certain relationships have been found which are interesting and perhaps significant

The specific gravity of the urine or of any solution containing more than one solute is the summation of the several specific gravities which would result if each solid were dissolved singly to the same volume of solution. In other words, each individual solid contributes an increment which varies with the concentration of the substance. This was confirmed by experiment for urea and sodium chloride, the substances of primary importance in these studies

At 15°C a 1 per cent solution of urea has a specific gravity of 1.0028, a 1 per cent solution of sodium chloride, 1.0067. In these studies the specific gravity of the urine was determined at 15° with the Westphal balance, the urea estimated by the urease method, the chloride by the Volhard method. The content of urea and chloride can then be converted into terms of specific gravity and these values analyzed. Thanks are due to Mr J G Camack for technical assistance

In these studies both single voidings and twenty-four hour collections were used. In the normals to be reported no effort to control diet was made and such variations in diet as occurred were spontaneous. The studies made upon normals on high or low salt or protein intake are as yet too few to report. In the nephritics studied the diets were shifted from low salt and protein to high salt and protein without altering the constancy of the relations about to be described

For convenience we may call that part of the total specific gravity which is not accounted for by the urea and chloride, the residual

<sup>1</sup> Read before the Association of American Physicians, May 7, 1924



specific gravity By plotting this fraction against the total specific gravity its percentage contribution to the whole can be made evident. If the ratio of urea plus chlorides to the other solids remains constant and the total specific gravity varies only with changes in the ratio of water to solids, then the values plotted will fall on the same percentage diagonal.

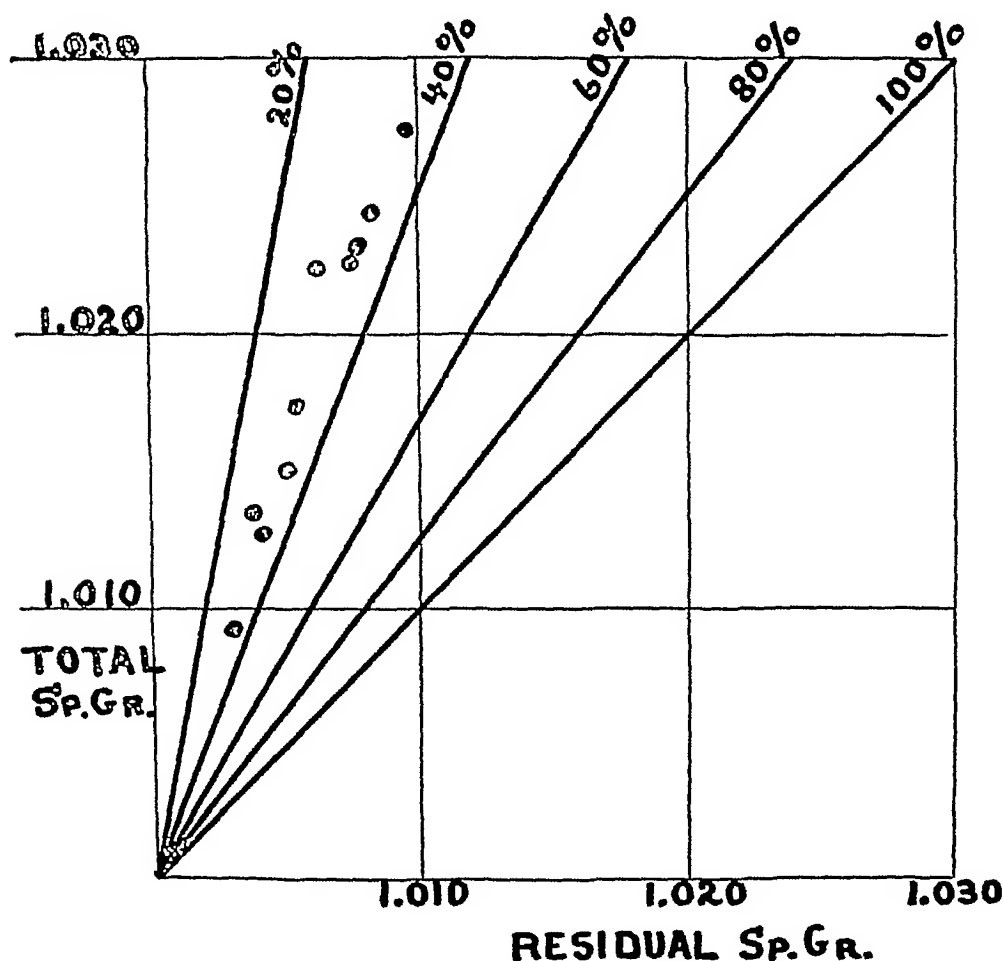


FIG 1

Ten specimens from a normal individual, taken over a period of a year and a half are plotted in this manner in figure 1. A marked constancy of ratio is evident.

In figure 2 specimens from another normal also fall along a percentage diagonal, a little to the right of the other. In this individual the residual specific gravity forms about 42 per cent of the total.

In figure 3 a group of thirty-six specimens from ten normals are plotted. In almost every instance the "residual" specific gravity is between 25 and 50 per cent. This constancy is striking when one considers the wide variation in total specific gravity, the number of individuals, and the uncontrolled diet.

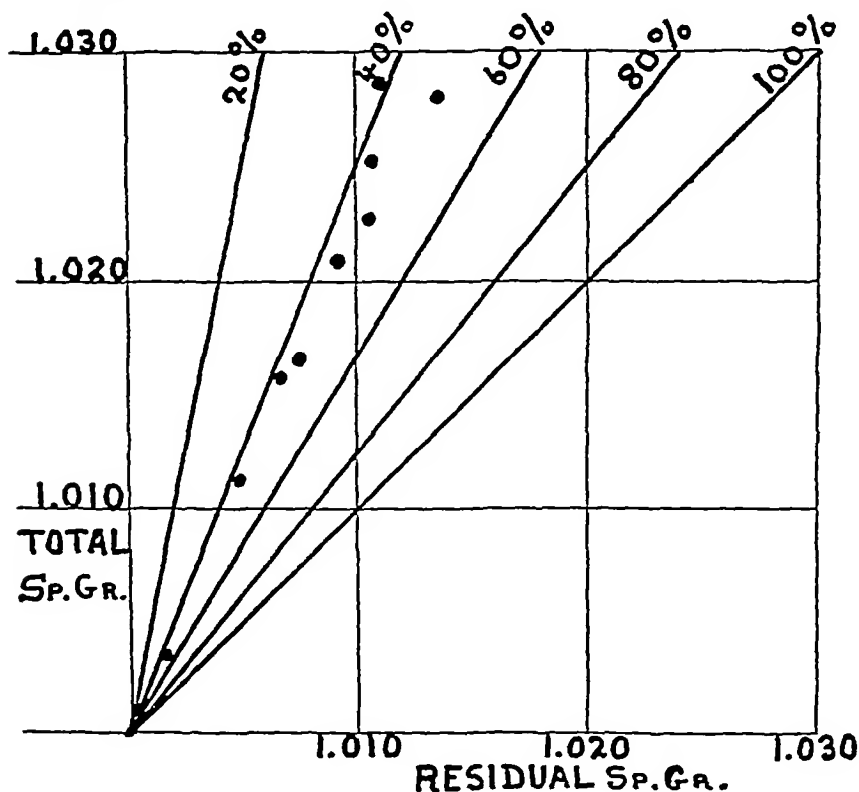


FIG 2

This group of normals is contrasted in figure 4 with twenty specimens from twelve patients with definite chronic glomerular nephritis. In this figure the residual percentage of specific gravity is plotted against the total specific gravity. The nephritic values indicated by crosses, exhibit a ratio of residual to total specific gravity between 43 and 75 per cent. This narrow variation is the more striking in that it persisted despite efforts to vary the intake of chloride and of protein.

In other words the solids other than the urea and chlorides make up a greater proportion of the total solids in nephritic than in normal urine. Confirmation of this is found in conductivity studies by Gram (1), working in the same laboratory, who found that in the urine of nephritics the non-chloride fraction of the electrolytes is relatively increased as compared with the chloride fraction.

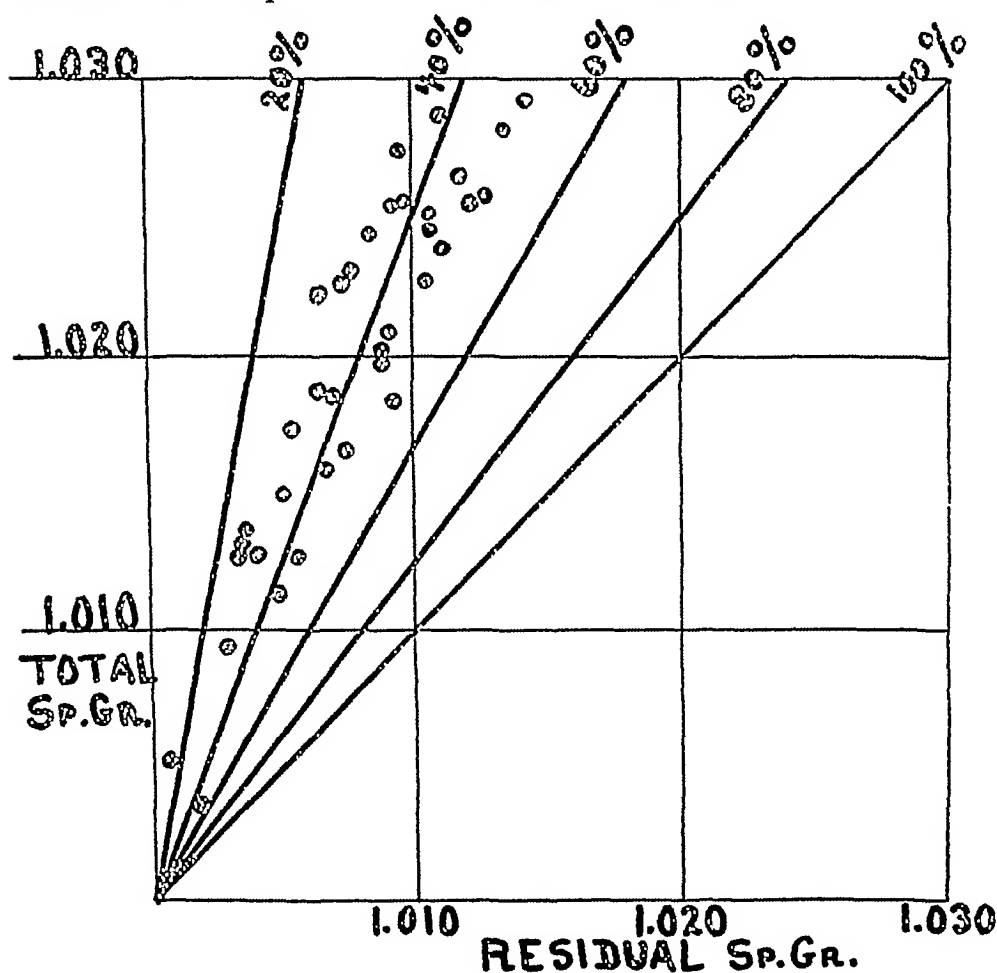


FIG. 3

Individual nephritics exhibit the same constancy of ratio as do individual normals.

The highest residual specific gravity percentages were obtained in a series of observations on a nephritic of the type with much edema. In this patient the blood pressure was normal, the 'phthalein elimination 40 per cent, the blood urea nitrogen 24 mg and the blood cholesterol

730 mg per 100 cc The twelve values obtained from this patient are plotted in figure 5 The residual specific gravity percentage lies with great constancy between 63 and 82 This constancy persisted in spite of changes from an almost salt free diet to a diet rich in salt,

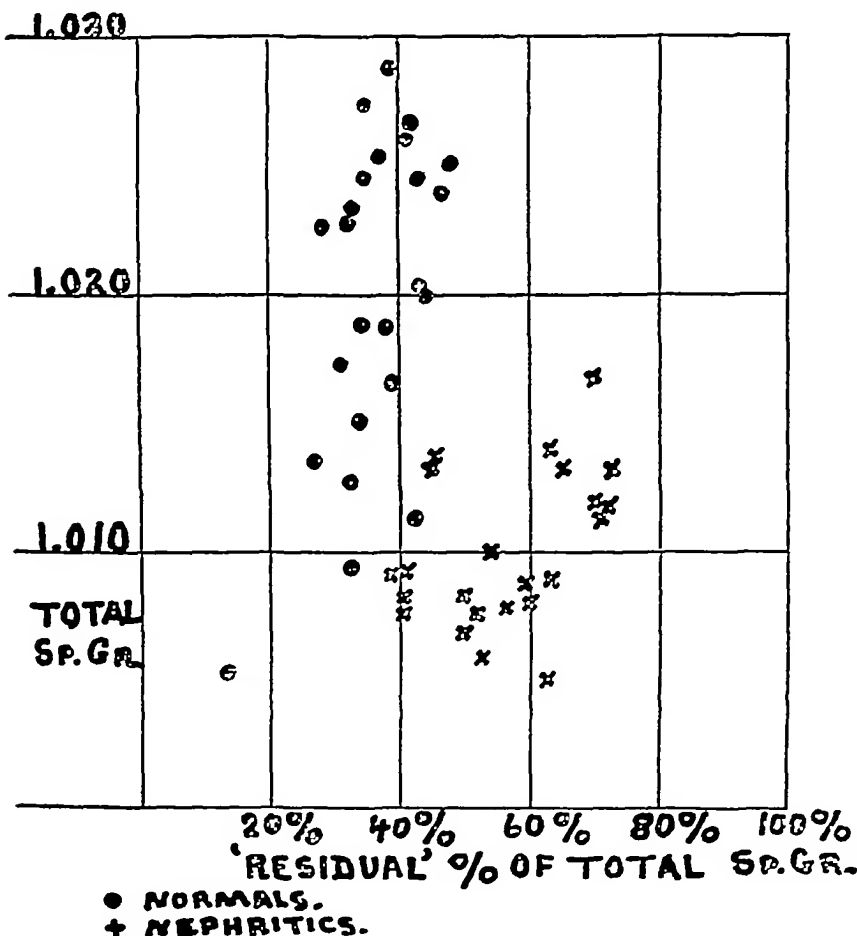


FIG 4

and from a diet low in protein to one high in protein The urine of this patient alone contained sufficient albumin to require consideration This was measured by the Esbach method, converted into terms of specific gravity and subtracted from the total A 1 per cent solution of albumin has a specific gravity of 1.0024

It is interesting to note that the so-called "conductivity chloride discrepancy" which Atchley, Loeb, Benedict and Palmer (2) described in the serum of nephritics was found in this type of edematous nephritis. While it is probable that this change in conductivity should be attributed largely to the altered protein content of the

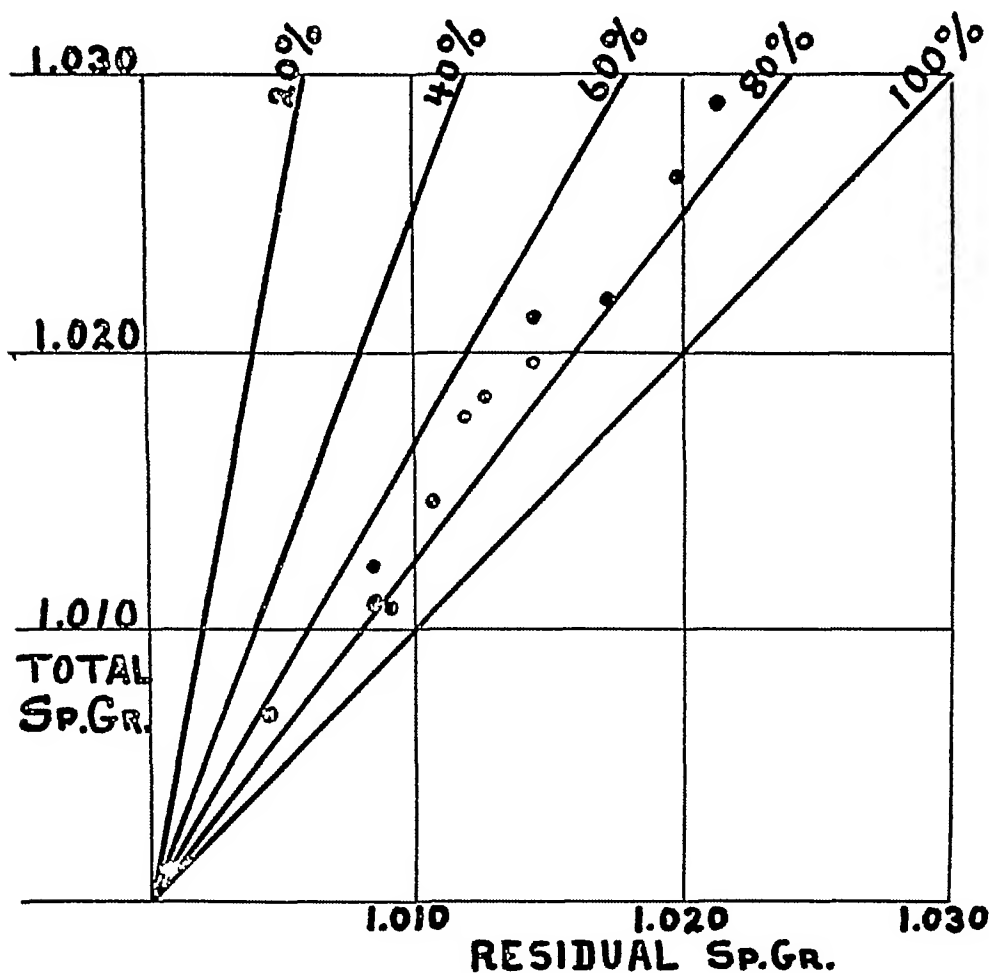


FIG 5

serum, nevertheless it is also probable that our findings in the urine in some way reflect the altered condition of the blood

From our observations it is clear that the nephritic fails to excrete as much urea and chlorides in a urine of a given specific gravity as would a normal. The urea plus chloride specific gravity percentage is remarkably constant in the several individuals and groups whether normal or nephritic, but the urea and chloride which form this fraction

vary considerably in relation to one another. In the nephritics as a rule it is the chloride specific gravity which is especially reduced even when liberal salt is given in the diet. But even in the nephritic there is a considerable inverse variation of the chloride and the urea. It is our intention to study further the influence of diet, the solids forming the non-urea non-chloride fraction of the specific gravity, and the osmotic pressure and other features of the urine.

#### SUMMARY

When the specific gravity of the urine is analyzed into a fraction due to urea plus chlorides and the residual fraction due to the other solids, exclusive of albumin, the following relations have been observed:

1. A marked constancy of ratio between these fractions in any individual, normal or nephritic, independent of diet.

2. In normals the residual fraction forms 25 to 50 per cent of the total.

3. In 32 observations on 13 nephritics the residual fraction formed 43 to 82 per cent of the total.

4. The nephritic, independent of diet, excretes less "urea plus chlorides" in a urine of a given specific gravity than would a normal.

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# ON THE RELATION BETWEEN CONDUCTIVITY AND CHLORIDES IN THE URINE

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Previous papers by Gram and Norgaard (1) and Gram (2) and also by Atchley, Loeb, Benedict and Palmer (3) have shown in both normal and most pathological human sera a marked constancy in the ratio of NaCl concentration to the NaCl equivalent of conductivity. This is expressed as the ratio  $\frac{[\text{NaCl}]}{\text{NaCl eq}}$ . A recent study by Pepper (4) has shown that in nephritis the relative proportion of non-chloride, non-urea constituents in the urine is increased.

It would seem that a determination of the ratio  $\frac{[\text{NaCl}]}{\text{NaCl eq}}$  in such urines would serve to throw light on whether this relative increase is due to non-chloride electrolytes or not.

In tables 1 and 2 we give the results of an examination of 11 normal and 11 nephritic urines respectively. All specimens were freshly voided and no ammoniacal decomposition had taken place. The ratio in normal urines of course is not nearly as fixed as in normal sera, but still has remained within the limits 0.84 and 0.63 in all cases, with an average of 0.726.

In nephritic urines on the other hand the ratio  $\frac{[\text{NaCl}]}{\text{NaCl eq}}$  was with one exception lower than in any of the normal urines, varying between 0.67 and 0.34 with an average of 0.524.

This marked discrepancy in the ratio cannot be attributed to the presence of albumin in the nephritic urines since the quantities of

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TABLE 1  
*Eleven normal urines*

Name	Date	NaCl eq of conduct	[NaCl]	Ratio [NaCl] NaCl eq
	<i>1923</i>	<i>per cent</i>	<i>per cent</i>	
H C G	April 13	1 85	1 43	0 72
G E C	April 14	0 59	0 39	0 66
J	April 14	2 16	1 81	0 84
J H A	April 21	1 89	1 31	0 69
D H M	April 21	1 73	1 09	0 63
L J	April 23	1 91	1 51	0 79
P	April 23	1 91	1 35	0 71
K	April 23	1 185	0 91	0 77
L	June 4	1 54	1 10	0 71
P P	June 18	1 56	1 02	0 65
C	September 10	1 96	1 61	0 82
Maximum				0 84
Minimum				0 63
Average				0 726

TABLE 2  
*Eleven nephritic urines*

Name	Diagnosis	Date	NaCl eq of conduct.	[NaCl]	Ratio [NaCl] NaCl eq
		<i>1923</i>	<i>per cent</i>	<i>per cent</i>	
N B	Glomerulo-nephritis	April 26	0 49	0 27	0 55
G B	Nephritis	May 21	0 53	0 23	0 43
P	Chronic nephritis	June 2	0 44	0 15	0 34
L G	Nephritis	June 6	0 36	0 20	0 56
J N	Nephritis	June 7	0 67	0 35	0 52
W V	Chronic nephritis	June 8	0 67	0 41	0 61
M	Nephritis	June 20	0 91	0 42	0 46
M T	Nephritis	June 20	0 845	0 47	0 56
S	Acute nephritis	June 20	0 73	0 43	0 59
A T	Chronic nephritis	September 5	0 51	0 34	0 67
S M	Chronic nephritis	September 5	0 425	0 20	0 47
Maximum					0 67
Minimum					0 34
Average					0 524

albumin are too small to affect materially the ratio, besides any such influence would cause an increased, not a decreased ratio

A lower concentration of non-electrolyte crystalloids, principally urea, would depress the ratio in nephritis but could not cause the difference observed, even if the urea decreased from 5 to 0 per cent.<sup>1</sup>

Only a few of these patients were placed on a diet poor in salts, so that this will not explain the decreased ratio  $\frac{[\text{NaCl}]}{\text{NaCl eq}}$

No exact classification was attempted in diagnosis, but the material included both acute and chronic nephritis and sclerotic kidneys in different stages of severity

#### SUMMARY

In nephritic urine the non-chloride electrolyte fraction is increased relative to the chloride fraction

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<sup>1</sup> To correct conductivity observed for urea we use the formula

$$C_c = C_o \frac{100}{100 - dx}$$

$C_c$  = corrected conductivity,  $C_o$  = observed conductivity,  $d$  = constant, which is 1.1 for urea,  $x$  = the percentage of urea



# BLOOD REACTION AND BLOOD GASES IN PNEUMONIA

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Peabody in 1912 (1) published a paper on the metabolism in pneumonia, in which he reviewed the previous literature and studied among other factors the blood gases and acid-base balance. Since Peabody, a number of other investigators have studied the blood gases and the question of the existence and importance of acidosis in pneumonia. The present paper is a report of observations on these subjects, in which recently developed methods have made possible the attainment of more complete results and apparently have justified the drawing of deductions more definite in some respects than those attainable from previous data.

Peabody stated that "the high excretion of ammonia and the low excretion of sodium chloride are two of the most characteristic features of the urine during fever. The diminution of the carbon dioxide of the blood is apparently a constant accompaniment of fever." These results indicated a shift of some degree towards acidosis in the acid-base balance, but whether it was sufficient to be of clinical significance was at the time uncertain. Peabody states, "The evidence points against the theory that the retention of sodium chloride in fever depends on acidosis."

In diabetic acidosis and in experimental poisoning by mineral acids there is not a retention of bases as there is in pneumonia." In the gases of the venous blood much greater variations in oxygen than in carbon dioxide were found.

Palmer (2) found that, although considerable amounts of a very weak unknown organic acid could be titrated in the urine in certain severe cases of pneumonia, the alkali reserve of the venous blood plasma, as determined by the  $\text{CO}_2$  capacity method of Van Slyke and Cullen (3) was uniformly normal or nearly so. Acidosis of metabolic origin was consequently excluded.

Stillman, Van Slyke, Cullen, and Fitz, (4), in a series of acidosis cases of which the rest were diabetic, published protocols on one case with a greatly diminished plasma  $\text{CO}_2$  capacity in acute nephritis *after* pneumonia. The alkali deficit, which was accompanied by an almost complete cessation of ammonia excretion (unpublished data) was apparently due to the pneumonic nephritis. It disappeared as the kidney function and ammonia excretion improved.

Stadie (5) and Stadie and Van Slyke (6) studied the oxygen content and capacity of the blood and the carbon dioxide content and capacity of the plasma in both venous and arterial blood of pneumonia patients. They confirmed Palmer's observation of normal alkali reserve as indicated by the  $\text{CO}_2$  capacities. From the gas contents of the blood they observed that "even when pulmonary conditions in pneumonia become so involved that the arterial blood is incompletely oxygenated, the arterial and venous carbon dioxide values are not increased above the usual normal levels." There was no evidence of pulmonary  $\text{CO}_2$  retention.

The data up to this point (1920) indicate that an acidosis of metabolic origin, such as would result in an alkali deficit in the body, is a rarity in pneumonia. Because the data do not include either determinations of the  $\text{CO}_2$  tension in the blood, or of the pH from which the  $\text{CO}_2$  tensions can be estimated, they do not exclude the possibility of a  $\text{CO}_2$  acidosis, due to hindrance in the evolution of  $\text{CO}_2$  from the pathologically involved lungs) although the combined determinations of  $\text{CO}_2$  content and  $\text{CO}_2$  capacity by Stadie and Van Slyke made a  $\text{CO}_2$  retention appear improbable.

Barach, Means and Woodwell (7) in 1922 estimated the pH of the arterial and venous blood in pneumonia by interpolation on the  $\text{CO}_2$  absorption curve, corrected for the observed oxygen unsaturation. They found the alkali reserve normal or slightly subnormal. In 3 of 10 patients, however, a low pH was observed which rose to normal after the crisis, the alkali reserve showing appreciable rise at the same time, although it had not been seriously lowered. The results indicated the possibility of a tendency towards both alkali deficit and lowered pH in some cases, and the authors suggested the use of alkali therapy in such cases.

Binger, Hastings and Neill (8), however, found that the continued use of even moderate amounts of bicarbonate in a case of pneumonia with somewhat diminished salt excreting power led to a dangerous alkalosis and edema, due to apparent inability to excrete the alkali.

It seemed advisable to make further observations on the blood changes in pneumonia patients, the more so because at the time of the work of Barach, Means and Woodwell, the colorimetric pH method for blood was not available, and their pH values were determined by a method which makes such demands on technique that a certain proportion of irregular results is difficult to avoid. Thirty observations of the pH and  $\text{CO}_2$  content, and 22 observations of the oxygen content and oxygen capacity of the arterial blood have accordingly been made on 16 patients.

In our observations the gases were determined on the Van Slyke (9) manometric apparatus. The pH's were determined colorimetrically by Cullen's method (10) in all cases, electrometrically in 12 cases, and by calculation from the CO<sub>2</sub> absorption curves in 2 cases. All precautions to collect and preserve the blood without change in its constituents were observed (Austin, Cullen, et al, 11)

### THE pH DETERMINATIONS

*Electrometric and colorimetric values*—In table 1 are given the results of the colorimetric determinations of pH at 20°C and the

TABLE 1

*The difference between the electrometric pH at 38° and the colorimetric pH at 20° of the arterial blood of pneumonia patients*

No	pH		$\Delta$ pH	No	pH		$\Delta$ pH
	Col. 20°	Elect. 38°			Col. 20°	Elect. 38°	
19	7.72	7.42	0.30	25	7.77	7.48	0.29
20	7.72	7.42	0.30	26	7.72	7.43	0.29
21	7.71	7.49	0.22	27	7.70	7.43	0.27
22	7.63	7.40	0.23	28	7.76	7.46	0.30
23	7.66	7.40	0.26	29	7.82	7.50	0.32
24	7.55	7.32	0.23	30	7.59	7.35	0.24

Average  $\Delta$ pH = 0.27

Average deviation = 0.03

Maximum deviation = 0.05

electrometric determinations made on the same blood at 38°C. The average difference,  $\Delta$ pH, between these determinations is 0.27 pH. The difference for the blood of normal individuals was found by Cullen to be 0.22 pH. This value has been confirmed in this laboratory. The reason for the difference between the  $\Delta$ pH found for normals and for pneumonia patients is unknown. The formula we have used in the calculation of the blood pH at 38° from the colorimetric pH at 20° is

$$\text{pH}_{38^\circ\text{C}} = \text{pH}_{20^\circ\text{C}} - 0.27$$

Since there were rather wide differences in the temperatures of the patients at the time the blood analyses were made, a correction was

applied to convert the pH at 38° to its corresponding value at the temperature of the patient. The temperature coefficient for the pH of blood is not well established. Direct determinations of the electrometric pH of serum at 38° and 20° have indicated that the  $\Delta\text{pH}$  per 1°C. is approximately 0.01. This value has been used to correct the pH to the temperature of the blood as drawn.

Recent determinations made in this laboratory indicate that by making the colorimetric pH determinations at the temperature of the patient, the necessity for temperature correction is avoided (Hastings and Sendroy, paper in press) but the technique for such determinations had not been developed when the present work was done.

TABLE 2

*A comparison of electrometric, colorimetric and calculated pH's*

	Electrometric pH	Colorimetric pH	Calculated pH
Normals			
1	—	7.38	7.40
2	7.39	7.41	7.38
Pneumonia patients			
9	—	7.44	7.44
10	7.42	7.47	7.47

*pH values calculated from the carbon dioxide absorption curve*  
 Since the pH results of Barach, Means and Woodwell (7) were all determined by calculation from the carbon dioxide content and the carbon dioxide tension, four experiments were performed in order to compare values obtained thus with those obtained by direct electrometric or colorimetric determinations.

Two of the experiments were on the venous blood of normal individuals and two were on the arterial blood of pneumonia patients. The comparisons between the pH values obtained by direct determination and by calculation are given in table 2. The agreement is within the limits of error of the method employed. Since difficulty has been experienced in obtaining satisfactory agreement by these two methods, certain points which contributed to the success of our experiments may be mentioned.

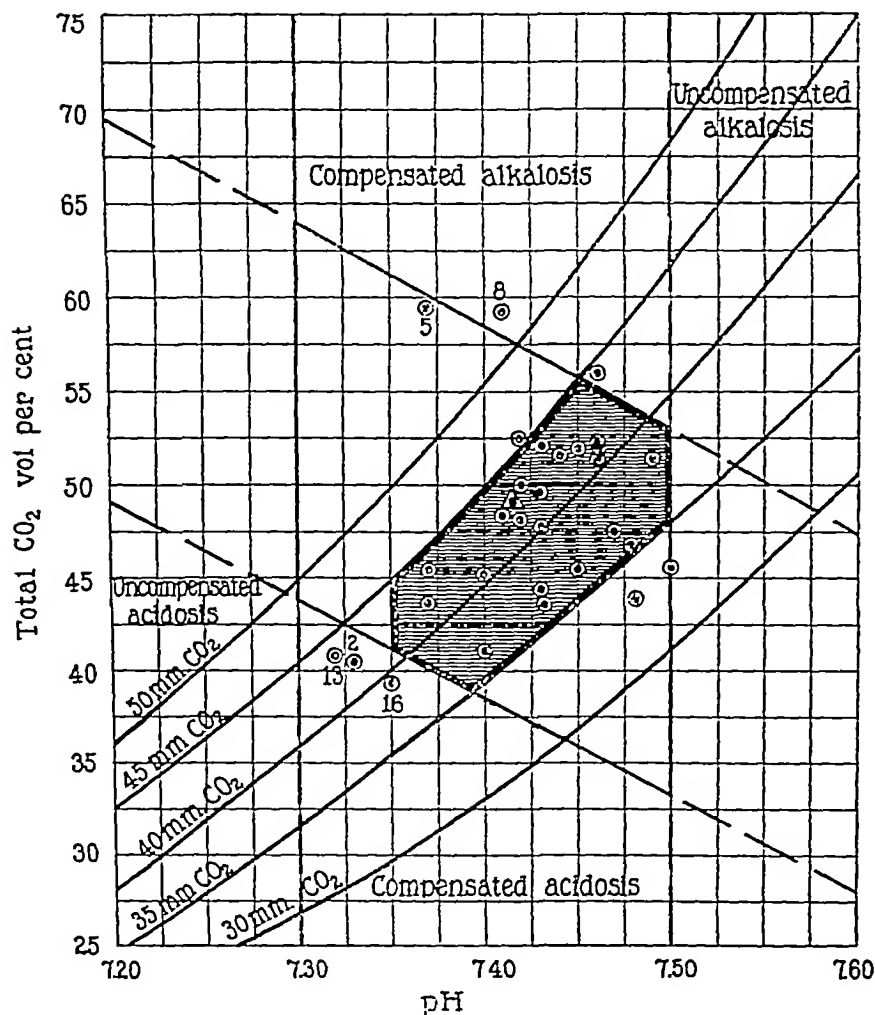


FIG 1 ACID-BASE DETERMINATIONS ON PNEUMONIA PATIENTS ARE PLOTTED WITH pH AS ABSCISSAE AND CO<sub>2</sub> CONTENT AS ORDINATES

Constant CO<sub>2</sub> tension lines run diagonally across the chart. The shaded area represents the revised normal acid-base area of arterial blood. The point  $\times$  is the average of a number of normal arterial acid-base values obtained by Barr and his collaborators.



*A summary of the hydrogen ion co.*

Determination number	Case number	History number	Date	Organism	Area of pulmonary involvement at time blood was drawn	Day of disease blood was drawn	Day of disease temperature became normal	Day of disease passed
1	1	4522	4-27-22	Pneumococcus Type III	Consolidation of left lower lobe, and whole right lung	9	11	
2	2	4597	10-17-22	Pneumococcus Type I	Consolidation of right middle lobe Resolution of right lower lobe	8		
3			10-23-22		Consolidation of right middle lobe Resolution of right lower lobe	14		
4			10-24-22		Consolidation of right middle lobe Resolution of right lower lobe	15		18
5	3	4619	11- 5-22	Pneumococcus Group IV	Consolidation of right middle and lower lobes	4	6	
6			11-10-22		Consolidation of right middle and lower lobes	9		
7	4	4623	11-10-22	Pneumococcus Group IV	Consolidation of left upper lobe Diffuse bronchitic râles	5		
8			11-13-22		Consolidation of left upper lobe. Diffuse bronchitic râles	8		
9			11-14-22		Consolidation the same. Diffuse râles less	9		
10			11-15 12		Consolidation the same. Diffuse râles less	10	10	12
11	5	4630	11-15-22	Pneumococcus Type III	Consolidation of left upper lobe. Râles left lower and right upper	4		
12			11-16-22		Consolidation of left upper and lower, and right upper lobes	5	5	
13			11-17-22		Consolidation of left upper and lower, and right upper lobes	6		6
14	6	4648	12-12-22	Pneumococcus Type III	Consolidation of right middle and lower lobes	5	12	
15	7	4651	12-18-22	Pneumococcus Type II	Consolidation of left upper and lower Diffuse bronchitic râles	5		6
16	8	4657	12-29-22	Pneumococcus Type I	Consolidation of left upper and lower, and right upper lobes	6		

## Analyses of the arterial blood in pneumonia

Remarks	Rectal temperature °C	pH at body temperature	Arterial blood analyses									
			CO <sub>2</sub> content				CO <sub>2</sub> tension mm Hg	O <sub>2</sub> content		O <sub>2</sub> capacity		Per cent saturation
			Whole blood		Plasma			mm	vol per cent	mm	vol per cent	
			mm	vol per cent	mm	vol per cent						
Respirations 58 per minute.	38.9	7.45	25.0	56.0	—	—	45.2	6.8	15.2	7.3	16.4	92.7
Atherosclerosis marked	38.8	7.45	23.2	52.0	—	—	43.8	6.7	15.1	8.2	18.4	82.1
Patient had received 600 cc. type I antipneumococcus serum before bleeding pneumococcus meningitis	38.5	7.46	23.3	52.3	—	—	42.7	—	—	—	—	—
	38.1	7.33	17.9	40.4	—	—	42.9	6.7	15.1	8.3	18.6	81.2
	38.7	7.45	20.4	45.7	—	—	38.1	—	—	—	—	—
	37.7	7.37	19.5	43.7	—	—	42.3	—	—	—	—	—
	39.6	7.42	19.9	44.6	—	—	39.6	6.8	15.2	—	—	—
	38.8	7.43	19.4	43.6	—	—	38.2	6.6	14.7	7.8	17.5	84.0
	38.1	7.41	21.5	48.3	—	—	43.1	7.1	15.9	—	—	—
	37.3	7.46	23.2	52.0	—	—	41.2	7.0	15.6	7.2	16.2	96.4
Patient died suddenly when afebrile and apparently convalescent	39.6	7.47	20.6	46.2	—	—	45.9	7.6	17.0	7.8	17.5	97.2
	39.0	7.36	20.4	45.7	—	—	46.2	5.8	13.1	8.6	19.2	68.2
	37.8	7.37	26.6	59.7	—	—	58.2	7.2	16.2	8.2	18.4	83.1
Temperature normal for short interval only on 5th day of disease	39.7	7.42	21.4	47.9	—	—	44.0	7.7	17.2	8.9	19.9	86.5
Right pleural effusion Hydropneumothorax	39.3	7.41	22.3	50.0	—	—	46.8	7.0	15.6	9.2	20.6	75.8
Patient had received 400 cc. type I antipneumococcus serum before bleeding	39.6	7.46	21.1	47.4	—	—	37.2	5.9	13.3	6.6	14.7	9.5

The blood was treated with one per cent neutral sodium fluoride as well as potassium oxalate which, as Lovatt Evans (12) has shown, inhibits acid formation during the period of saturation. Further,

Determination number	Case number	History number	Date	Organism	Area of pulmonary involvement at time blood was drawn	Day of disease blood was drawn	Day of disease temperature became normal	Day of disease patient died
17			12-30-22		Consolidation of left upper and lower, and right upper lobes	7	12	S
18	9	4671	1-19-23	Pneumococcus Group IV	Consolidation of right middle and lower lobes	5	6	
19			1-23-23		Consolidation of right middle and lower lobes	7	6	
20	10	4670	1-20-23	Pneumococcus Group IV	Consolidation of right upper lobe	4	6	
21	11	4691	2- 7-23	Pneumococcus Group IV Streptococcus hemolyticus	Consolidation right lower lobe Right pleural effusion Rales over left lower lobe	9		26
22	12	4690	2- 7-23	Pneumococcus Group IV	Consolidation of right lower lobe	5	6	
23	13	4708	3- 1-23	Pneumococcus Type II	Consolidation of left lower lobe	7	11	
24			3- 6-23		Consolidation of left lower lobe	12		
25	14	4710	3- 2-23	Pneumococcus Group IV	Consolidation of right middle and lower lobes	4	8	
26			3- 7-23		Signs of resolution over entire right lung	9		
27	15	4715	3- 6-23	Pneumococcus Group IV	Consolidation of right middle and lower lobes	3	9	
28			3- 7-23		Consolidation of right middle and lower lobes	4		
29			3-14-23		Resolution of right middle and lower lobes	11		
30	16	4729	3-27-23	Pneumococcus Group IV	Consolidation of whole right lung and left lower lobe Diffuse bronchitic rales	7		7 O

e indicates electrometric determination

the CO<sub>2</sub> saturation curves were determined on both the reduced and oxygenated blood, thereby permitting the proper correction to be made for the unsaturation of the blood. Finally, the pK' used in

calculating the pH values was obtained by taking into consideration the pH, the concentration of total hemoglobin, and the degree of saturation of the blood (fig 6 b of Van Slyke, Wu, McLean, 13)

Continued

Remarks	Rectal temperature		Arterial blood analyses									
	°C	pH at body temperature	CO <sub>2</sub> content				CO <sub>2</sub> tension mm Hg	O <sub>2</sub> content		O <sub>2</sub> capacity		Per cent saturation
			Whole blood		Plasma			mm Hg	vol per cent	mm Hg	vol per cent	
			mm	vol per cent	mm	vol per cent						
Oxygen discontinued during bleeding	37.5	7.41	26.5	59.3	—	—	50.8	4.5	10.0	6.9	15.5	64.6
	39.3	7.44	23.0	51.6	25.9	58.0	42.6	5.9	13.3	6.3	14.1	94.4
	37.2	7.43e	23.5	52.8	27.5	61.7	45.8	7.1	15.9	7.3	16.4	97.0
	39.1	7.42e	21.4	48.1	26.3	59.0	45.3	8.7	19.5	9.5	21.3	91.6
Streptococcus hemolyticus Bronchopneumonia. Septicemia (Streptococcus hemolyticus) Death	39.6	7.48e	23.9	51.4	27.7	62.0	53.5	7.7	17.3	8.6	19.2	90.2
	38.2	7.40e	20.2	45.2	25.2	56.4	41.5	—	—	—	—	
	40.2	7.39e	18.4	41.2	21.7	48.7	38.7	—	—	—	—	
	37.2	7.33e	18.2	40.8	—	—	40.8	5.1	11.4	5.7	12.8	89.7
Lead to right upper lobe occurred	39.7	7.47e	19.5	43.6	23.4	52.4	35.3	7.9	17.7	8.2	18.4	96.2
	37.5	7.43e	22.2	49.7	—	—	42.5	7.4	16.6	8.1	18.1	91.8
Uremia retention	39.6	7.42e	23.3	52.2	—	—	45.2	4.7	10.6	7.0	15.6	68.0
	40.2	7.45e	23.2	52.0	—	—	43.2	5.8	13.0	7.2	16.1	80.8
	37.4	7.51e	20.4	45.7	24.5	55.1	32.6	6.2	13.8	7.4	16.5	83.7
Complicated by marked albuminuria with urea retention	40.0	7.34e	17.5	39.3	—	—	41.8	—	—	—	—	

The protocols of these experiments and the figures showing the results are given in tables 5 and 6, and figures 3 and 4

## RESULTS

The experimental results are collected in table 3 and are graphically shown in the acid-base diagram, figure 1

*The pH of the blood* Among the 30 observations on pneumonia patients there was no pH lower than 7.30 or higher than 7.50 (Where both colorimetric and electrometric values were available, the latter were taken as the more accurate) Since the pH limits of 7.30 and 7.50 for normals have been confirmed in this laboratory, it may be said that no pneumonia patient studied by us had a pH definitely outside the normal limits Certainly there was no case of uncompensated acidosis among them

*The CO<sub>2</sub> content of the blood* Values of the CO<sub>2</sub> content of arterial blood of normal resting individuals have been determined by Barr (14) The average of these determinations is 22 millimols per liter (49.4 volumes per cent)

The CO<sub>2</sub> contents of the blood of the pneumonia patients are about equally distributed on either side of the mean line Except for three high and three low values they lie within the original limits of the normal area outlined by Van Slyke (15) Some of these cases require special mention Of the low ones, No. 2 had a pneumococcus meningitis and died three days later, No. 16 was complicated by nephritis and died the day the observation was made, No. 13 was afebrile at the time of the analyses Of the high points, No. 5 was moribund and died on the day of the observation, No. 8 had been having oxygen therapy by nasal catheter The fact that this was discontinued during the bleeding may have contributed to the marked unsaturation of the blood

It is concluded from determinations of CO<sub>2</sub> content taken in conjunction with those of the pH, that an acidosis, either compensated or uncompensated, is rarely, if ever, encountered in pneumonia uncomplicated by other abnormal conditions

*The CO<sub>2</sub> tension* Twenty-five of the thirty arterial CO<sub>2</sub> tensions, calculated from the serum pH and CO<sub>2</sub> content, were within the 35 to 45 mm iso-pressure lines of figure 1 The CO<sub>2</sub> tension was calculated from the CO<sub>2</sub> content and plasma pH by formula 5 in table 4 of Austin et al (11), viz

$$p_{\text{CO}_2} = \frac{[\text{CO}_2]}{0.0587\alpha_{\text{CO}_2}(1 + 10^{\text{pH} - \text{pK}'})}$$

$[\text{CO}_2]$  = volume per cent total  $\text{CO}_2$  in whole blood

$\alpha_{\text{CO}_2}$  = solubility coefficient of  $\text{CO}_2$  in whole blood

$\text{pK}' = \text{pK}'$  of Hasselbalch's equation for whole blood,  $\text{pH} = \text{pK}' + \log \frac{[\text{HCO}_3]}{[\text{H}_2\text{CO}_3]}$

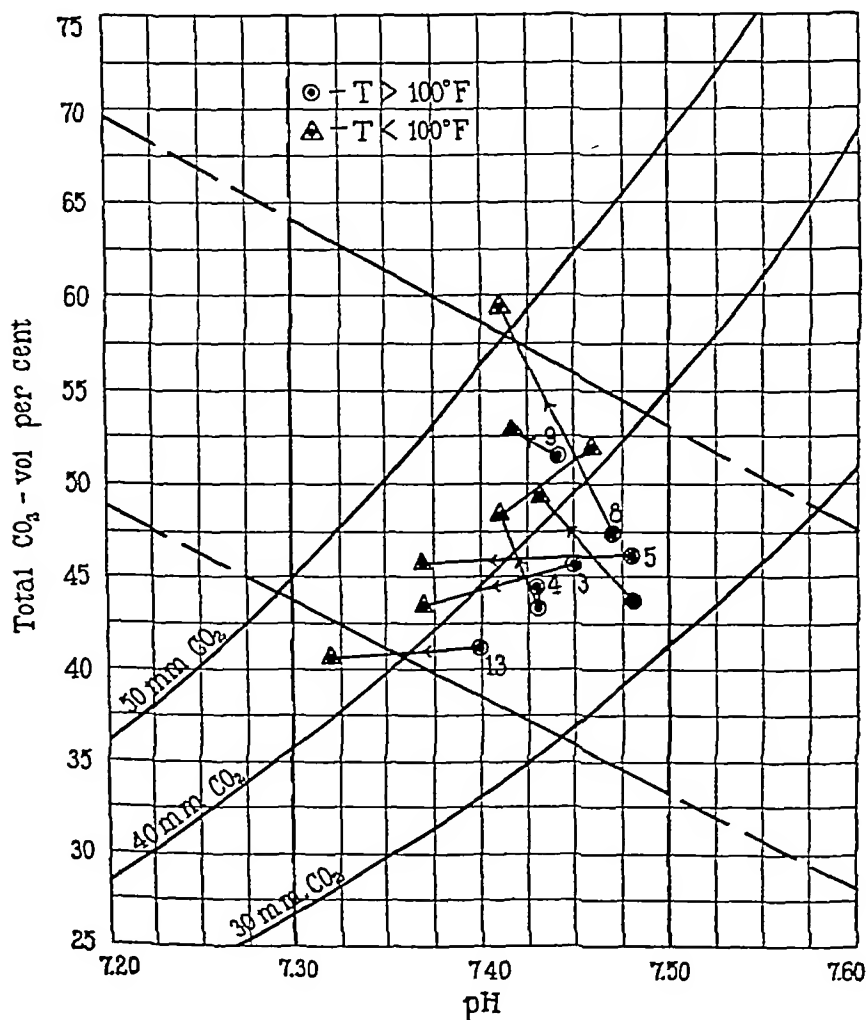


FIG 2 ACID-BASE DETERMINATIONS OBTAINED ON PNEUMONIA PATIENTS DURING AND AFTER THE FEBRILE PERIOD

The value of  $\alpha_{\text{co}_2}$  was estimated to be proportional to the water content of the blood (13) The water content, for blood of varying hemoglobin content, is calculated from Equation 30 of Van Slyke, Wu, and McLean, (13) as

$$\text{cc. H}_2\text{O per cc blood} \approx 0.94 - 0.0067 \text{ Hb}$$

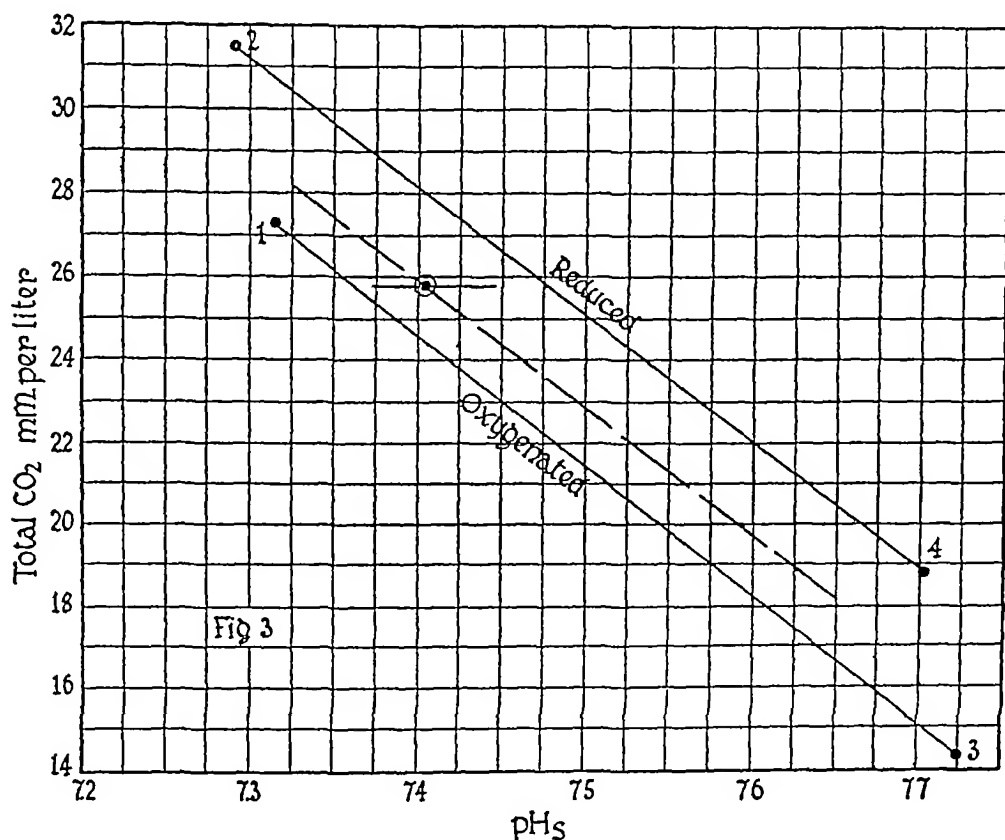


FIG 3 JANUARY 9, 1923  $\text{CO}_2$  ABSORPTION CURVES OF OXYGENATED AND REDUCED BLOOD OF A NORMAL INDIVIDUAL PLOTTED WITH pH AS ABSCISSAE AND  $\text{CO}_2$  CONTENT AS ORDINATES

The point within the  $\circ$  represents the acid-base balance of the venous blood as drawn determined by the  $\text{CO}_2$  content and degree of unsaturation The pH thus estimated is 7.40, as determined colorimetrically, 7.38

Hb = hemoglobin content of blood in volume per cent of oxygen capacity (The constants in their equation are here altered to change the  $\text{H}_2\text{O}$  from terms of weight to those of volume, and the Hb from terms of millimols to those of volumes per cent oxygen capacity)

Since the solubility coefficient of  $\text{CO}_2$  for water is 0.555, the coefficient for blood is estimated as

$$\alpha_{\text{CO}_2} = 0.555 (0.94 - 0.0067 \text{ Hb})$$

The  $pK'$  value for whole blood, as shown by Warburg (16) by Van Slyke, Wu, and McLean (13) and by Peters, Bulger, and Eisemann

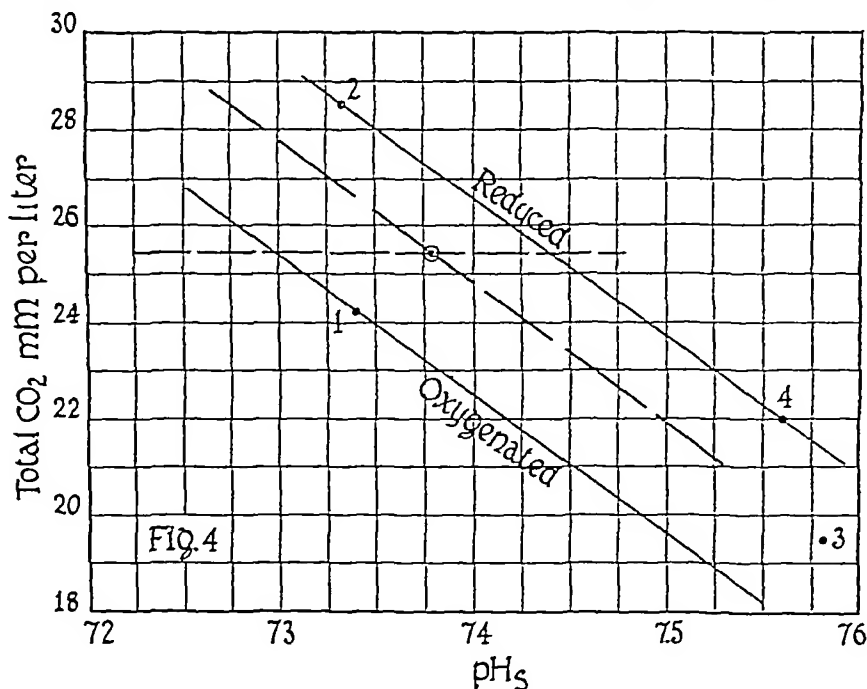


FIG 4 JANUARY 25, 1923  $\text{CO}_2$  ABSORPTION CURVES OF OXYGENATED AND REDUCED BLOOD OF A NORMAL INDIVIDUAL PLOTTED WITH pH AS ABSCISSAE AND  $\text{CO}_2$  CONTENT AS ORDINATES

The point within the  $\circ$  represents the acid-base balance of the venous blood as drawn determined by the  $\text{CO}_2$  content and degree of unsaturation. The pH thus estimated is 7.38, as determined colorimetrically 7.41, electrometrically, 7.39

(17), is greater for whole blood than for plasma by an amount,  $\Delta pK'$ , dependent on the oxygen saturation, pH, and hemoglobin content of the blood. Hence, for whole blood,

$$pK' = pK'_s + \Delta pK',$$



where

$$pK'_s = pK' \text{ for serum} = 6.12$$

$\Delta pK'$  is estimated from figure 6 *b* of Van Slyke, Wu, and McLean, with an added correction for oxygen unsaturation

With the above values for  $\alpha_{\text{CO}_2}$  and  $pK'$  in whole blood, the formula relating  $p_{\text{CO}_2}$  to  $[\text{CO}_2]$  and  $pH_s$  becomes

$$p_{\text{CO}_2} = \frac{[\text{CO}_2]}{0.0326 (0.94 - 0.0067 Hb) (1 + 10^{pH - pK'_s - \Delta pK'})}$$

TABLE 4

*The CO<sub>2</sub> tension of the arterial blood during and after the febrile period*

Case No	During febrile period			After febrile period		
	CO <sub>2</sub> tension	Per cent saturation with oxygen	Temperature	CO <sub>2</sub> tension	Per cent saturation with oxygen	Temperature
	mm Hg		°C	mm Hg		°C
3	38.1	—	38.7	42.3	—	37.7
4	39.6	—	39.6	41.2	96.0	37.3
	38.2	84.0	38.8			
	43.1	—	38.1			
5	45.9	97.2	39.6	58.2	88.1	37.8
	46.2	68.2	39.0			
8	37.2	90.5	39.6	50.8	64.6	37.5
9	42.8	94.4	39.3	45.8	97.0	37.2
13	38.7	—	40.2	40.8	89.7	37.2
14	35.3	96.2	39.7	42.5	91.8	37.5
15	45.2	68.0	39.6	32.6	83.7	37.4
	43.2	81.0	40.2			

The progress of the CO<sub>2</sub> tension during the course of the disease is shown in eight cases in which data during and after the febrile period were obtained. These results are shown in table 4 and figure 2. It is seen that there is a lower CO<sub>2</sub> tension during the febrile period than after. Whether this is the result of the increased temperature alone or whether it had its origin in the local pulmonary lesion it is impossible to say. Haggard (18) found that elevation of the body temperature by immersion in hot baths was sufficient to lower the alveolar CO<sub>2</sub> tension and reduce the dissolved CO<sub>2</sub> in the blood, but Fridericia (19) who studied the alveolar CO<sub>2</sub> tension in febrile diseases, believes

that increased temperature alone is insufficient to account for the lower tension. That anoxemia *per se* is not the causal agent in the lowered arterial  $\text{CO}_2$  tension is suggested by the fact that the unsaturation of the arterial blood in some cases did not seem to be less in the period of low tension than in the subsequent afebrile period.

That the respiratory mechanism is unimpaired in its ability to maintain a normal  $\text{CO}_2$  tension and blood reaction is evidenced by the fact

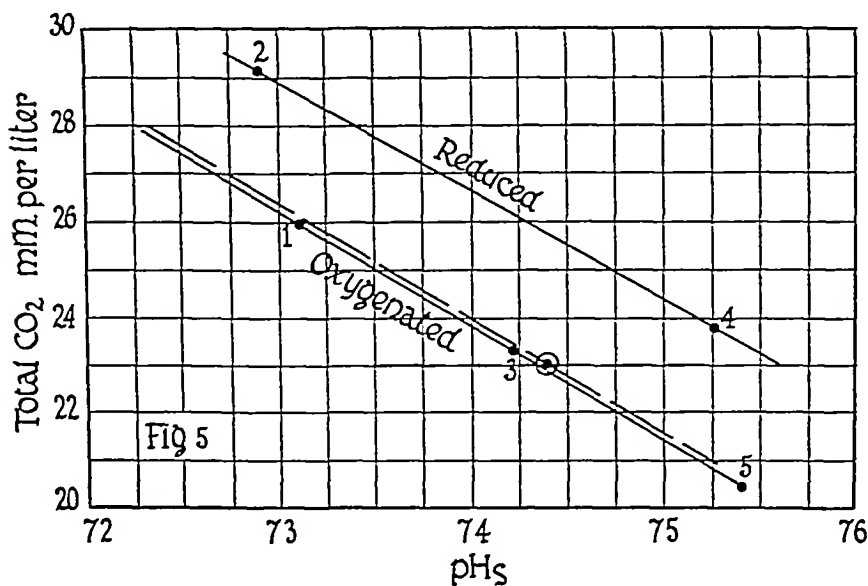


FIG 5 JANUARY 19, 1923  $\text{CO}_2$  ABSORPTION CURVES OF OXYGENATED AND REDUCED BLOOD OF A PNEUMONIA PATIENT PLOTTED WITH pH AS ABSCISSAE AND  $\text{CO}_2$  CONTENT AS ORDINATES

The point within the  $\circ$  represents the acid-base balance of the arterial blood as drawn determined by the  $\text{CO}_2$  content and degree of unsaturation. The pH thus estimated is 7.44, as determined colorimetrically, 7.44.

that in only 2 of the 30 analyses was the arterial  $\text{CO}_2$  tension found above 45 mm., and in no case was the pH below 7.30. There was no tendency towards  $\text{CO}_2$  acidosis.

The effect of the lung changes in pneumonia on the oxygenation of the arterial blood on the other hand is significant. Of the 10 cases in which the oxygen saturation of the arterial blood was determined,

8 showed on one or more occasions arterial saturation below 90 percent, which figure is probably lower than occurs in any normal person at rest at sea level. In 6 cases arterial saturation below 85 per cent was noted, a level of arterial saturation at which symptoms of mountain sickness may begin in normal individuals who are transferred to high altitudes (20)

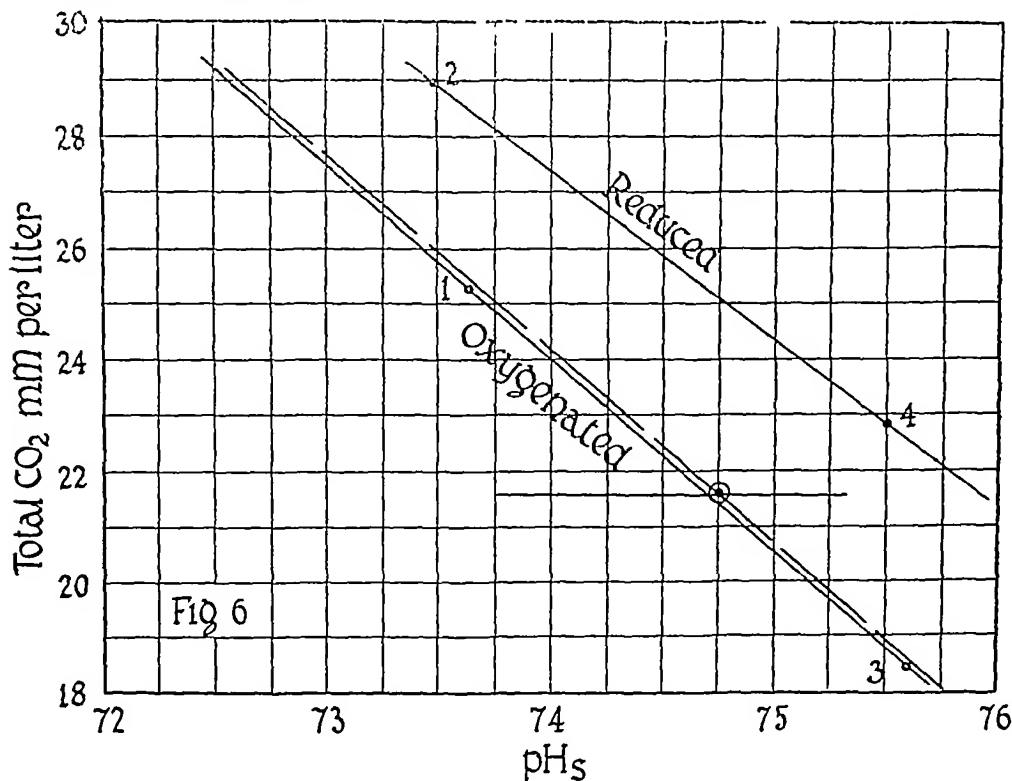


FIG 6 JANUARY 20, 1923 CO<sub>2</sub> ABSORPTION CURVES OF OXYGENATED AND REDUCED BLOOD OF A PNEUMONIA PATIENT, PLOTTED WITH pH AS ABSCISSAE AND CO<sub>2</sub> CONTENT AS ORDINATES

The point within the  $\circ$  represents the acid-base balance of the arterial blood as drawn, determined by the CO<sub>2</sub> content and degree of unsaturation. The pH thus estimated is 7.47, as determined colorimetrically, 7.47, electrometrically, 7.42.

Various known facts indicate that the oxygenation of the blood in the lungs is much more susceptible to failure than is the removal of CO<sub>2</sub>. Krogh (21) has shown that CO<sub>2</sub> diffuses through animal membranes 30 times faster than O<sub>2</sub>. Henderson (22) has calculated that

TABLE 5

*A comparison of the pH of normal human blood determined directly and calculated from the CO<sub>2</sub> saturation curves. Blood treated with 0.4 per cent K<sub>2</sub>CO<sub>3</sub> + 0.1 per cent NaF*

Normal, No 1

Date January 9, 1924

pK' = 6.12 + ΔpK'

H<sub>2</sub>CO<sub>3</sub> = 0.0271 pCO<sub>2</sub>

Analysis of blood after saturation with definite CO<sub>2</sub> and O<sub>2</sub> tensions

No.	pO <sub>2</sub>	HbO <sub>2</sub>	pCO <sub>2</sub>	H <sub>2</sub> CO <sub>3</sub>	Total CO <sub>2</sub>	BHCO <sub>3</sub>	log $\frac{BHCO_3}{H_2CO_3}$	pK'	pH
	mm	mm	mm	mm	mm	mm			
1	(135)	9.18	66.1	1.79	27.28	25.49	1.154	6.165	7.319
2	(0)	0.11	79.5	2.15	31.50	29.35	1.135	6.154	7.289
3	(135)	9.23	15.0	0.41	14.53	14.12	1.537	6.190	7.727
4	(0)	0.15	20.0	0.54	18.88	18.34	1.531	6.173	7.704

Analysis of blood as drawn

Total Hb + HbO <sub>2</sub>	HbO <sub>2</sub>	$\frac{HbO_2}{Hb + HbO_2}$	Total CO <sub>2</sub>	pH
mm	mm	per cent	mm	
(9.40)	5.97	63.0	25.76	7.40—calculated 7.38—colorimetric

Normal, No 2

Date January 25, 1923

pK' = 6.12 + ΔpK'

H<sub>2</sub>CO<sub>3</sub> = 0.0270 pCO<sub>2</sub>

Analysis of blood after saturation with definite CO<sub>2</sub> and O<sub>2</sub> tensions

No	pO <sub>2</sub>	HbO <sub>2</sub>	pCO <sub>2</sub>	H <sub>2</sub> CO <sub>3</sub>	Total CO <sub>2</sub>	BHCO <sub>3</sub>	log $\frac{BHCO_3}{H_2CO_3}$	pK	pH
	mm	mm	mm	mm	mm	mm			
1	(135)	9.34	56.2	1.52	24.27	22.75	1.175	6.165	7.340
2	(0)	0.38	65.9	1.78	28.53	26.75	1.177	6.155	7.332
3	(135)	9.38	27.4	0.74	19.48	18.74	1.404	6.178	7.582*
4	(0)	0.49	32.0	0.86	22.00	21.14	1.392	6.168	7.560

Analysis of blood as drawn

Total Hb + HbO <sub>2</sub>	HbO <sub>2</sub>	$\frac{HbO_2}{Hb + HbO_2}$	Total CO <sub>2</sub>	pH
mm	mm	per cent	mm	
9.62	4.39	46	25.59	7.38—calculated 7.39—electrometric 7.41—colorimetric

\* Not used in plotting oxygenated curve.

TABLE 6

*A comparison of the pH of pneumonia patients' blood determined directly and calculated from the CO<sub>2</sub> titration curves*

Patient, No 9

Date January 19, 1923

$pK' = 6.12 + \Delta pK'$

$H_2CO_3 = 0.0280 pCO_2$

Analysis of blood after saturation with definite CO<sub>2</sub> and O<sub>2</sub> tensions

No	pO <sub>2</sub>	HbO <sub>2</sub>	pCO <sub>2</sub>	H <sub>2</sub> CO <sub>3</sub>	Total CO <sub>2</sub>	BHCO <sub>3</sub>	log $\frac{BHCO_3}{H_2CO_3}$	pK'	pH
	mm	mm.	mm	mm	mm	mm			
1	(135)	6.60	57.8	1.62	25.88	24.26	1.175	6.147	7.312
2	(0)	0.57	68.5	1.92	29.17	27.25	1.152	6.141	7.293
3	(135)	6.87	42.1	1.18	23.27	22.09	1.272	6.150	7.422
4	(0)	0.45	33.9	0.95	23.76	22.81	1.380	6.146	7.526
5	(135)	6.73	28.6	0.80	20.32	19.52	1.388	6.156	7.544

Analysis of blood as drawn

Total Hb + HbO <sub>2</sub>	HbO <sub>2</sub>	Per cent saturation	Total CO <sub>2</sub>	pH
mm	mm		mm	
6.90	5.92	94.4	23.02	7.44—calculated 7.44—colorimetric

Patient No 10

Date January 20, 1923

$pK' = 6.12 + \Delta pK'$

$H_2CO_3 = 0.0270 pCO_2$

Analysis of blood after saturation with definite CO<sub>2</sub> and O<sub>2</sub> tensions

No	pO <sub>2</sub>	HbO <sub>2</sub>	pCO <sub>2</sub>	H <sub>2</sub> CO <sub>3</sub>	Total CO <sub>2</sub>	BHCO <sub>3</sub>	log $\frac{BHCO_3}{H_2CO_3}$	pK'	pH
	mm	mm	mm	mm	mm	mm			
1	(135)		56.8	1.53	25.31	23.78	1.191	6.168	7.359
2	(0)	0.25	65.1	1.76	28.88	27.12	1.188	6.158	7.346
3	(135)	9.19	27.4	0.74	18.45	17.71	1.380	6.178	7.558
4	(0)	0.51	33.3	0.90	22.72	21.82	1.385	6.167	7.552

Analysis of blood as drawn

Total Hb + HbO <sub>2</sub>	HbO <sub>2</sub>	Per cent saturation	Total CO <sub>2</sub>	pH
mm	mm		mm	
9.51	8.69	91.4	21.45	7.47—calculated 7.42—electrometric 7.47—colorimetric

the blood attains practically the  $\text{CO}_2$  tension of the alveolar air by the time it has passed through about half the length of a lung capillary, while the arterial blood after traversing completely the pulmonary capillaries still has an oxygen tension about 25 millimeters lower than the alveolar air. It has furthermore been shown by various observers that the respiratory mechanism shows a relatively weak response to oxygen lack as compared with that to carbon dioxide excess. Accumulation of  $\text{CO}_2$  may cause a man to increase his ventilation per minute by 1000 per cent, while the maximum response to oxygen lack is about 50 per cent. Furthermore, if part of the lung area is cut off from air, it is possible by over ventilation of the remainder to keep the  $\text{CO}_2$  tension of the mixed arterial blood down to normal, but since only 20 volumes per cent of oxygen can be held by the average blood, it is not possible to overcharge one portion of the pulmonary blood with oxygen in order to compensate for under oxygenation in another. These considerations would lead to the conclusion that, in any individual breathing the ordinary atmosphere, respiratory hindrance must result in serious anoxemia long before  $\text{CO}_2$  acidosis has become at all significant.

It will be noted in figures 1 and 2 that the shaded area indicated as normal is bounded not only by normal pH and alkali reserve lines, but also by the 35 and 45 mm  $\text{CO}_2$  tension lines within which the alveolar tensions of most normal individuals lie. It appears from results at present available that respiration is stimulated by high  $\text{CO}_2$  tension of itself, as well as by the high  $[\text{H}^+]$  it may cause, and that the condition found in the blood is a resultant of the two effects.

#### SUMMARY

1 A study has been made of the pH,  $\text{CO}_2$  content,  $\text{CO}_2$  tension, oxygen content, and oxygen capacity of the arterial blood in pneumonia. The  $\text{CO}_2$  tension was calculated from the pH and  $\text{CO}_2$  content of the blood. The other values were all determined directly.

2 A comparison was made of colorimetric and electrometric pH values with values calculated from the  $\text{CO}_2$  absorption curves. A fair degree of consistency was obtained.

3 A lower arterial  $\text{CO}_2$  tension during the febrile period than after the return to normal temperature was noted in 7 cases. Oxygen

unsaturation and lowered  $\text{CO}_2$  tension do not occur together with sufficient regularity to indicate a causal relationship. Such relationship between temperature and  $\text{CO}_2$  tension seems more probable.

4 No tendency towards an acidosis of either metabolic or respiratory origin was noted. The alkali reserve was within or near normal limits in every case. The pH likewise was in each instance within normal limits, 7.30 to 7.50, with the greater number of observations in the more alkaline half of this range.

5 These results contra-indicated alkali therapy in all the pneumonia cases studied.

6 In 8 of the 10 cases in which the arterial oxygen saturation was determined, an abnormally low saturation was observed at some stage of the disease. Taken with the non-occurrence of  $\text{CO}_2$  acidosis, these results support the conclusion, made probable by known physiological and physico-chemical data, that when the mechanism for gas exchange in the lungs is affected absorption of oxygen fails before elimination of carbon dioxide is significantly impaired.

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# A METHOD FOR THE DETERMINATION OF THE AMOUNT OF OXYGEN AND CARBON DIOXIDE IN THE MIXED VENOUS BLOOD OF MAN

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## INTRODUCTION

The exchange of gases between the blood and the fixed tissues of the body is essential for life. It is accomplished by a complex mechanism adapted to meet the ever changing conditions of the cells of the animal organism. Quantitative studies of this vital phenomenon are of importance because knowledge of the amount of oxygen lost or of carbon dioxide accumulated by a unit of blood in its passage through the body is not only in itself significant but also allows the calculation of the output of the heart per minute according to the principle of Fick (1870). When the heart rate is also known, the output of the heart per beat may be calculated, and this, Yandell Henderson states, is probably both for physiological and clinical purposes the most important quantitative function of the body. The study reported in this paper was initiated with the purpose of measuring the output of the heart.

Calculation of the cardiac output by Fick's principle demands a knowledge of two facts: (1) the amount of oxygen absorbed by the body per minute and (2) the amount of oxygen taken up by each unit of blood as it passes through the lungs. Division of the total oxygen per minute by the oxygen taken up by each unit of blood will express the number of units of blood passing through the lungs each minute. The determination of the oxygen taken up by each unit of blood requires knowledge of (1) the oxygen content of the blood as it leaves the lungs (arterial blood) and (2) the oxygen content of the blood as it enters the lungs (mixed venous blood).

Fick's principle may be applied as well if the corresponding figures for carbon dioxide are known.

## GASEOUS CONTENT OF THE MIXED VENOUS BLOOD

In lower animals arterial blood and true venous blood are obtained by direct puncture of the left and right ventricles but this method can not be applied to man. Arterial blood can be obtained safely and conveniently in human subjects by artery puncture, as described by Stadie (1919). To obtain mixed venous blood is, however, less simple, since there is abundant evidence to show that blood from a superficial vein may have a gas content quite different from that of the blood which, collected from the entire body, finally enters the lungs as the mixed venous blood (Uyeno and Doi (1922), Barcroft and Nagahaski (1921), Meakins and Davies (1922), Dautrebande, Davies and Meakins (1923), Peters, Barr and Rule (1921), Meakins, Dautrebande and Fetter (1923), Stewart (1923)).

It is necessary, therefore, that the measurement of the gas content of the mixed venous blood be approached indirectly. The problem may be approached by way of the lungs, using a respiratory method of the sort devised in the laboratory of Pflüger, who first suggested the use of the lungs as an aerotonometer. When blood comes in contact with air in the lungs, the gases in the blood and in the lung air tend to come into pressure equilibrium. If a gas mixture is held in the lungs, it takes up gases from the blood or gives them up to the blood according to whether the gas tensions in the mixture are lower or higher than those of the blood entering the lungs. This principle has withstood the attack initiated by Bohr (Barcroft, 1914), and seems firmly established. It is generally accepted that blood exposed at a given temperature to a given gas tension will contain the same amount of gas whether in a tonometer or in the lungs.

The tension of oxygen and of carbon dioxide in the mixed venous blood may be determined by the analysis of air after its equilibration in the lungs with the blood entering them. The tensions being known, the contents may be calculated by applying the tensions to the dissociation curves of the two gases for the blood of the individual subject. Upon these principles several methods for the determination of the gases of the mixed venous blood have been evolved, which overcome the various technical difficulties with more or less success. The contributions of Loewy and v Schrotter (1905), Plesch (1909), Henderson and Prince (1914), Meakins and Davies (1922), Christiansen, Douglas and Haldane (1914), Douglas and Haldane (1922), Fridericia (1918), Barcroft, Roughton and Shoji (1921), and Redfield, Bock and Meakins (1922) are especially noteworthy.

Reviews have recently been published by Henderson (1923) and by Wiggers (1923), and further mention of previous methods will be made therefore only as they are related directly to the one we shall describe.

The basic principle utilized by all previous methods is the determination by respiratory methods of the tension of oxygen or carbon dioxide in the mixed venous blood, and then the calculation by means of dissociation curves of the gas content of the blood at the tension determined. It has been shown that the amount of these gases taken up by blood when exposed to them at given tensions varies with different individuals, and that the tension of each gas involved influences the

dissociation curve of the other. Furthermore, when the physiological processes are disturbed by the changes incident to disease the dissociation curves may be no longer constant for the individual and should be determined at the time the gas tensions are determined. Therefore it appears that the application of gas tensions to dissociation curves not only entails a considerable technical burden but is open to the criticism of being indirect and perhaps untrustworthy in diseased subjects. We have devised a method in which the application of dissociation curves is not made.

### THE METHOD

The general principles of the method we have devised are as follows. By a respiratory method a gas mixture is obtained in which both the oxygen and the carbon dioxide are in equilibrium with these gases of the mixed venous blood. A tonometer is filled directly with this gas and blood of the subject is equilibrated with it under standard conditions. Blood so treated is, as it were, "artificial" mixed venous blood and its analysis supplies the data of which we are in search. The respiratory procedure is readily accomplished by untrained subjects under conditions suitable for the study of hospital patients.

*The gas tension of the blood.* We attacked the problem of obtaining the desired gas mixture by utilizing the method of Henderson and Prince. A rubber bag was filled with about 2,000 cc of air, and this was inspired by the subject after a deep expiration. Following the recommendation of Laurens (1918) the air was breathed back and forth several times within a period of ten seconds. After the third expiration into the bag it was closed by turning the tap, and a sample of the gas taken. After an interval of three minutes the process was repeated, the series of samples thus obtained represented the original contents of the bag modified during each period by the mixture of the residual lung air and contact with the blood flowing through the lungs. The samples were analysed in duplicate for oxygen and carbon dioxide with the Henderson modification of the Haldane apparatus.

In such a series of samples the  $\text{CO}_2$  tension usually became fixed after 4 to 6 rebreathings at about 45 mm and did not thereafter change no matter how many times rebreathed. The oxygen tension became fixed more slowly, the number of rebreathings necessary depending upon the composition of the gas mixture originally in the

bag, but eventually (after 6 to 9 rebreathings) it showed no further change, but remained at about 80 mm. It was evident that although the  $\text{CO}_2$  of the gas mixture might be in equilibrium with that of the mixed venous blood the oxygen was not, as with an oxygen tension of 80 mm the venous blood would be over 90 per cent saturated, which is obviously not the case. Such a rebreathing method is not to be expected to give an oxygen tension in equilibrium with the venous blood, as between each rebreathing period, room air containing approximately 21 per cent of oxygen is drawn into the lungs, and at least 1,000 cc of residual air containing 16 per cent oxygen remains in the lungs at the beginning of each rebreathing period. It is clear, as Plesch and others have pointed out, that before the contents of the bag are taken into the lungs a preliminary adjustment of the lung air must be made which reduces the oxygen to a point near that of the venous blood, in order to permit the oxygen of the lung air to come into equilibrium with that of the venous blood during the short time available for the exposure of the air to the blood. This reduction of the oxygen of the lung air was accomplished by taking two breaths of a low-oxygen mixture just before each rebreathing. This mixture was made up so that the lung air after two full breaths of the mixture had  $\text{CO}_2$  and oxygen tensions approximating those of the mixed venous blood. The samples of lung air were obtained by means of a Haldane tube into which was expired the second breath of the low-oxygen diluting mixture.

When each rebreathing was preceded by such a preliminary adjustment of the lung air, it was found that the fixation of oxygen tension occurred at a point not incompatible with previously known physiological facts and that this point varied but slightly in successive determinations on the same individual.

It was then necessary to devise a method for determining the amount of oxygen in the mixture which should be breathed in order to reduce the lung air to approximately that of the venous blood, and to test the assumption that a constant tension was reached which actually represented the oxygen tension of the mixed venous blood. The method that was used for this purpose consisted in carrying on simultaneously two similar experiments, using two large spirometers containing gas mixtures of somewhat different oxygen tensions, so adjusted that the

lung air obtained after two inspirations from one spirometer had a higher oxygen tension than that of the supposed venous blood, while the lung air obtained after two inspirations from the other spirometer had a lower oxygen tension than that of the supposed venous blood. The two rebreathing bags were used alternately, so that conditions were the same throughout all rebreathing periods. Each bag was filled at the onset with an expiration following two full inspirations from the corresponding spirometer, and then at three minute intervals, the air in one or the other bag was rebreathed three times following two full inspirations from one or the other spirometer. When the spirometers contained properly adjusted gas mixtures, the successive samples taken from one bag showed a lower oxygen tension after such rebreathing down to a point where no further change occurred, while the successive samples from the other bag showed a higher oxygen

TABLE 1

	Spirometer I (O <sub>2</sub> tension)	Spirometer II (O <sub>2</sub> tension)
	mm Hg	mm. Hg
Spirometer mixture	10.4	6.8
Alveolar sample	32.0	27.6
9th rebreathing	31.4	30.9

tension after each rebreathing up to a point where no further change occurred. The points where the successive samples remained constant were almost identical in the samples from the two bags. It was evident, therefore, that the oxygen tension in the two bags at the end of such a procedure was that of the mixed venous blood. A large number of such experiments were conducted on the same individual under fairly constant conditions of rest in order to determine not only the most suitable oxygen mixture but also the rapidity and accuracy with which the oxygen of the lung air came into equilibrium with the oxygen of the mixed venous blood. Table 1 gives the results of such an experiment. These figures together with other evidence that is not published furnish evidence that the oxygen tension of the gas mixture in the rebreathing bag may be brought into equilibrium with that of the blood entering the lungs when the oxygen of the lung air is properly diluted before each rebreathing. It was

found that the proper dilution of the oxygen of the lung air was brought about by inspiring a mixture containing from 0.7 to 2 per cent oxygen.

However, the two inspirations for reducing the oxygen also reduced the carbon dioxide of the lung air, so that the final carbon dioxide figure in the bag was too low. It was evident that if it was necessary to breathe a low oxygen mixture to reduce the oxygen it was necessary to breathe a high  $\text{CO}_2$  mixture to maintain the  $\text{CO}_2$  tension. It was found that when the diluting mixture contained 6 to 7 per cent  $\text{CO}_2$  the lung air after two diluting breaths had a  $\text{CO}_2$  tension of about 45 mm Hg and gave final mixtures in the bag which were only slightly lower than obtained by the method of Henderson and Prince. This slight discrepancy is assumed to be due to the difference in the oxygen saturation of the blood.

The gas mixture for adjusting the lung air was made up by introducing commercial nitrogen, room air, and carbon dioxide in a 100 liter spirometer. It was found important to mix the gases after they were run into the spirometer in order to get a constant mixture during experiments. Because of changes which occur in the gas mixture on standing over the water in the spirometer, it was made up immediately before it was used, and samples for analysis were withdrawn immediately after the rebreathing procedure.

*The respiratory procedure* The method of carrying out the respiratory procedure was as follows. All data were obtained under constant conditions of bodily exercise, of the taking of food, of room temperature and as far as possible of psychic activity. The subject was placed in a reclining chair and rested for a period of about half an hour and until the heart rate became slowed to 72 beats per minute or less. The apparatus was arranged as shown in figure 1. The breathing tube was adjusted so that it was exactly at the level of the subject's mouth, and only a slight movement of the head was necessary to remove the mouth from the apparatus. No other movements were allowed during the experiment. (In the last few experiments an additional 3-way cock has been attached to the breathing tube, so that the subject could remain connected to the apparatus by a rubber mouth-piece and still breathe from room air, spirometer, or bag as occasion demanded.) The tube from the spirometer was attached to a respiratory valve which in turn was attached directly to one arm

of a 3-way stop-cock. The rebreathing bag was attached to the other arm, so arranged that by turning the cock, the subject was in connection either with the spirometer or with the rebreathing bag. When all was adjusted the subject inspired two or three times from the spirometer, thus filling all tubes with the gas mixture. The last expiration was directed by the operator into the rebreathing bag by turning the stop-cock at the end of the last inspiration from the spirometer the bag having been previously empty. The tape of

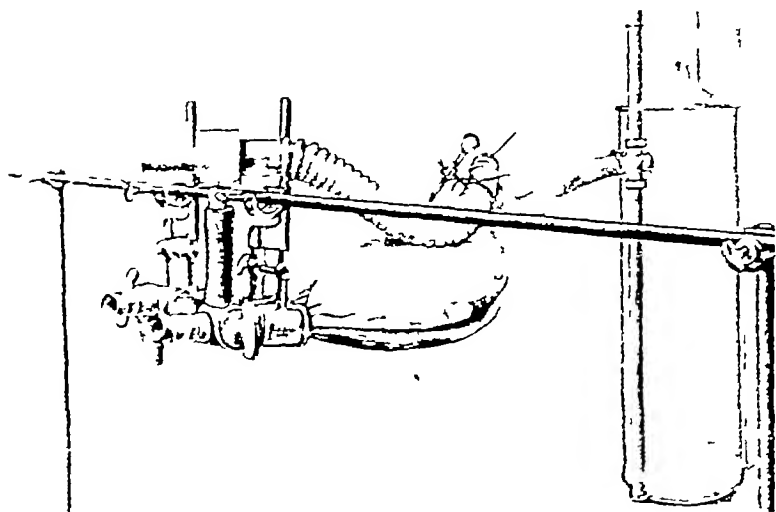


FIG 1 THE APPARATUS FOR CARRYING OUT THE RESPIRATORY PROCEDURE

The stop cock of the Haldane-Priestly tube is also shown

the spirometer was then read in order to record the amount of the gas mixture used each time for adjusting the lung air and the subject was encouraged to keep this amount fairly constant. As a rule about 60 per cent of the subject's vital capacity in each of the two respirations was found satisfactory. The fact that slight variations in the depth of the inspirations did not materially influence the rebreathing results was repeatedly demonstrated.

A typical respiratory procedure may be described as follows. The nose of the subject is closed with a nose clip and he applies his



mouth to the breathing tube and expires fully, the cock being turned so that the expiration escapes to the outside air. He then takes five full breaths, the stop-cock being turned at the end of the expiration following the second inspiration from the spirometer, so that the third, fourth and fifth breaths are in and out of the bag. At the end of the last expiration into the bag the cock is again turned, shutting off the bag, and the subject is directed to move the mouth from the breathing tube and to breathe outside air. The entire procedure requires from eighteen to twenty seconds, divided equally between the inspirations from the spirometer and the rebreathing to and from the bag. The procedure can be carried out correctly by ordinary hospital patients with little or no practice. The subject is fairly dyspnoeic for five or six breaths after the rebreathing and may show moderate cyanosis, but there is no change in heart rate. This procedure is repeated seven to eleven times, with an interval of three minutes between each. When samples are required they are taken from the bag immediately after each rebreathing so that diffusion of gases through the rubber bag plays no part in the results.

In order to demonstrate the changes that take place in the  $\text{CO}_2$  and oxygen tensions during each rebreathing a series of analyses was made. The spirometer contained at the end of the experiment a mixture having a  $\text{CO}_2$  tension of 45.6 mm (6.45 per cent) and an oxygen tension of 6.7 mm (0.94 per cent). The rebreathing bag contained 2000 cc of room air at the start. The successive samples gave the results shown in the curves of figure 2.

Two questions may be raised regarding the statement that the gases in the final gas mixture in the rebreathing bag are in equilibrium with the gases of true mixed venous blood. The first question is: do the samples withdrawn from the bag represent the gas mixture actually in contact with the blood flowing through the lungs? In other words, has there been a perfect mixture throughout the system composed of the lungs, respiratory passages, bag and the connecting tube? This question is best answered by reference to the recent work of Lundsgaard and Schierbeck (1923). They studied by an ingenious method the mixing of hydrogen and oxygen in a lung-bag system, and found that when two liters of a gas mixture were rebreathed three times following expiration to the residual air, a perfect mixture was obtained.

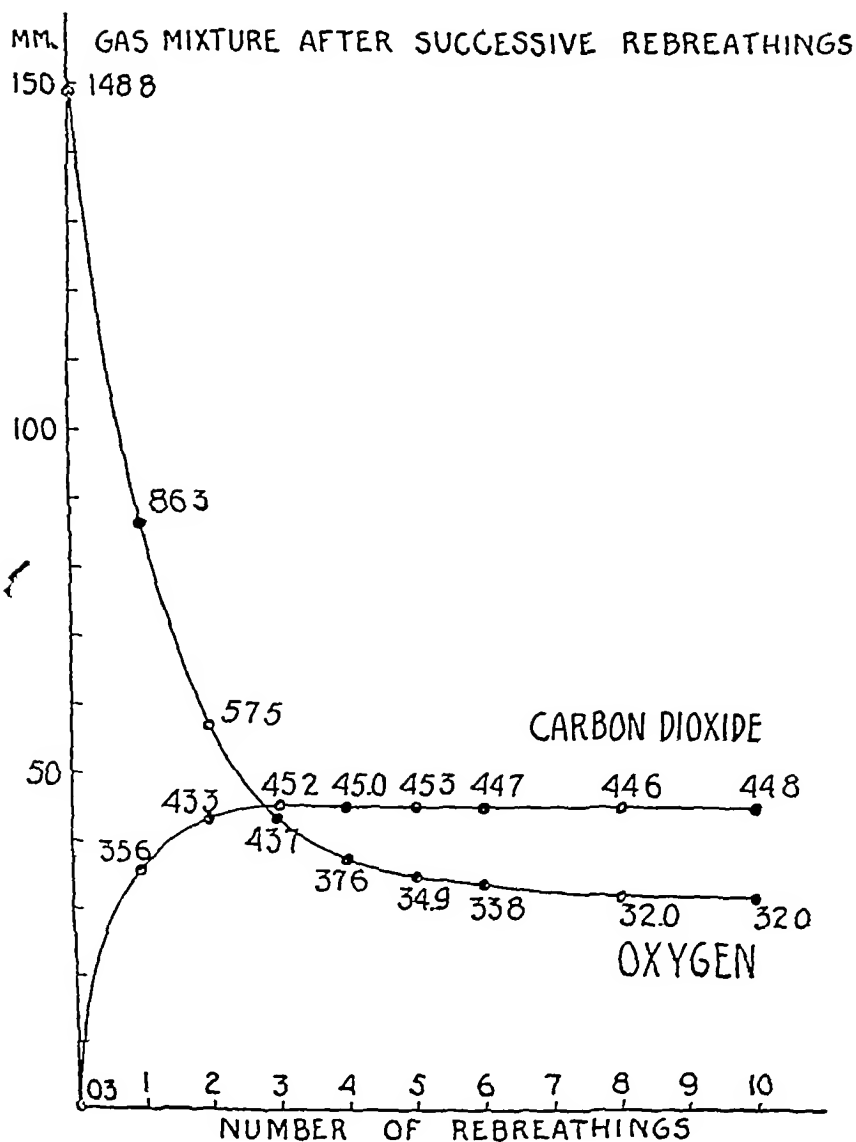


FIG 2 CURVE CONSTRUCTED FROM THE ANALYSES OF SUCCESSIVE SAMPLES WITHDRAWN FROM THE REBREATHING BAG AFTER EACH REBREATHING  
The figures give the O and CO<sub>2</sub> tensions obtained by each analysis

in both of the two normal subjects studied Douglas and Haldane (1922) have also considered this question Their experiments showed that by merely taking a single deep breath of a gas mixture it is impossible to produce an even mixture in the lung alveoli When, however, there is no great relative difference between the percentage of a gas in the mixture inspired and the percentage already present in the alveolar air, this source of fallacy is of minor importance They found that with a large difference in the percentages as in measurements of venous oxygen tension by their method at least three maximal breaths were required to obtain a perfect mixture Our subjects rebreathed on an average 2.25 liters of air to and from the bag, the composition of which was fairly close to that contained in the lungs after two inspirations from the spirometer We believe therefore that in the light of the constancy of our results and the experience of others that the gas samples from the bag represented exactly or very closely the mixture that had been exposed to the blood in the lungs It is possible that in studying patients with respiratory or circulatory disorders true mixtures may not be so readily obtained

The second question is may the blood flowing through the lungs during the rebreathing be considered as true mixed venous blood of a resting subject? There are two factors which may so alter the blood that it is not true venous blood One is improper aeration of the blood previous to its passage through the systemic capillaries, and the other is a change in rate of flow of blood through the systemic capillaries In order that the first factor may not come into play, the respiratory procedure is limited in time, so that no blood that has passed through the lungs when filled with other than the normal lung contents again returns to the lungs during the procedure Our own experiments and the experience of all other workers in this field are in agreement that for resting subjects no such error is introduced when the respiratory procedure does not exceed twenty seconds In regard to the rate of flow through the systemic capillaries, it is known that when the blood flow is slowed, more oxygen is given off and more  $\text{CO}_2$  taken up by the blood than when the blood is flowing at its usual rate It is probable that there is a certain amount of quickening of blood flow through the capillaries during our respiratory procedure, as during deep breathing blood is drawn more rapidly to the

heart than during normal respiration. During the two deep inspirations from the spirometer therefore, it is probable that the blood flow is quickened, and this may result in a more rapid flow of blood through some of the systemic capillaries. It would be expected that under these circumstances a slight rise in the oxygen tension and a slight fall in the  $\text{CO}_2$  tension of the mixed venous blood would occur, as less oxygen would be given up to the tissues and less  $\text{CO}_2$  taken away when the blood flow through the capillaries was quickened. This would in turn produce an error in the final blood gas figures which would diminish the figures for oxygen utilization and for  $\text{CO}_2$  production. This possible source of error was pointed out by Barcroft, Roughton and Shoji (1921), who thought that the alteration of the rate of blood flow through the systemic capillaries produced by their respiratory method introduced a trivial error in their results. We have not been able to investigate this possible source of error directly on our own subjects, but when our figures for oxygen utilization and  $\text{CO}_2$  production are compared with those of others, we find that there is no evidence of any diminution in the oxygen utilization or  $\text{CO}_2$  production. Our figures, as will be seen later, are usually higher than those obtained for these values by other workers in the field.

We believe therefore that all the questions regarding the proper mixing of gases and possible changes in the mixed venous blood may be answered satisfactorily, and that our respiratory method yields a gas mixture the gas tensions of which are within 10 mm Hg of the gas tensions of the blood.

*The gas content of the blood* In order to determine the gas content of the mixed venous blood two 300 cc tonometers were filled with the gas from the bag over mercury after the last rebreathing. Blood was then obtained under oil, oxalated and chilled. Approximately 6 cc of this blood was transferred to each tonometer and they were then rotated by motor for twenty minutes in a water bath at body temperature. The excess pressure in the tonometer due to the rise in temperature was released after five minutes of rotation by opening one end of the tonometer just under the surface of the water. In most of the experiments venous blood was used for equilibration, but following a suggestion of Peters (1924), arterial blood was used also in some later ones. No difference in the gas content of the blood was observed.

We have followed the first method described by Austin, Cullen, Hastings, McLean, Peters, and Van Slyke (1922) in equilibrating the blood. The blood was withdrawn into small sampling tubes from the tonometer while it was still immersed in the water bath, placed under oil or over mercury and kept in an ice bath until analysed. Samples of the gas mixture were taken from the tonometers after

TABLE 2

*Experiment 1*

May 29, 1923   Barometer 758.0   Temperature 25°C   Subject H   Weight 66 kilos  
Age 28   Average pulse rate 61

		Oxygen tension	Carbon dioxide tension
		<i>mm Hg</i>	<i>mm Hg</i>
Diluting mixture		28.2	45.0
Pulmonary air after dilution		38.7	45.4
Gas in rebreathing bag			
After 9th rebreathing		36.7	45.7
After 11th rebreathing		36.0	46.1
Gas in tonometer		35.8	45.9
	Oxygen content	Carbon dioxide content	Oxygen saturation
	<i>vol per cent</i>	<i>vol per cent</i>	<i>per cent</i>
Arterial blood	22.46	43.59	96
Mixed venous blood	16.41	48.58	71
Difference	6.05	4.99	25
	<i>cc</i>	<i>cc</i>	
Total gas exchange per minute	231	182	
Circulatory minute volume	3820	3650	
Output per beat	63	60	
Respiratory quotient, external	0.79		
Respiratory quotient, internal	0.83		

the blood was removed. The blood was analysed in the large model apparatus as described by Van Slyke and Stadie (1921), 1 cc of blood being used for each analysis, and results were accepted only when the duplicate analyses checked properly. A specimen of blood was withdrawn from the brachial artery immediately after the venous blood was obtained, following the technique described by Stadie (1919), and subjected to analysis under the same conditions as the equi-

brated blood The subject was kept at the same state of rest while the blood samples were being withdrawn as when the respiratory procedures were being carried on

## RESULTS

Our complete experiments were carried in the following order The subject came to the laboratory in the morning without breakfast,

TABLE 3

*Experiment 2*

June 5, 1923 Barometer 759.4 Temperature 28°C Subject H Weight 66 kilos  
Age 28 Average pulse rate 64

		Oxygen tension	Carbon dioxide tension
		mm. Hg	mm. Hg
Diluting mixture		8.2	53.3
Pulmonary air after dilution		39.1	45.4
Gas in rebreathing bag			
After 9th rebreathing		39.1	47.2
After 10th rebreathing		38.9	46.9
Gas in tonometer		38.8	46.9
	Oxygen content	Carbon dioxide content	Oxygen saturation
	vol. per cent	vol. per cent	per cent
Arterial blood	22.60	45.02	96
Mixed venous blood	17.01	49.52	73
Difference	5.59	4.50	23
	cc.	cc.	
Total gas exchange per minute	230	187	
Circulatory minute volume	4115	4150	
Output per beat	64	65	
Respiratory quotient, external	0.81		
Respiratory quotient, internal	0.80		

and rested in a reclining chair for half an hour, and until the pulse rate had assumed a constant low level (60 to 72 per minute) The expired air was collected during a six-minute period in a Tissot spirometer to determine the amount of oxygen absorbed and CO<sub>2</sub> produced per minute The respiratory procedure was then conducted as described As soon as this was completed, venous and arterial

blood samples were obtained, and the subject released. These procedures usually required about an hour and a half or two hours.

We wish to report as examples the results of two experiments carried out on a normal subject under similar conditions of complete rest during the post-absorptive period.

These experiments were performed on the same subject one week apart. They are in substantial agreement throughout. The last seems to be the more successful, as there is almost exact agreement of the respiratory quotients obtained from the blood gas analysis and from the gas exchange in the lungs (Tables 2 and 3).

It will be observed that in these experiments the circulatory minute volume calculated from the oxygen figures are in closer agreement than those calculated from the carbon dioxide figures. It may be said that in making a series of observations on the same subject the oxygen figures are much the more constant and that when great fluctuations occur they occur only in the carbon dioxide figures. This is understandable when it is considered that in determining the blood flow from the difference between the arterial and venous oxygen in normal people there is, practically speaking, only one variable since the arterial oxygen content is fixed at approximately 95 per cent of the oxygen capacity, while when the flow is derived from the carbon dioxide content of arterial and venous blood we are dealing with two variables, since the arterial  $\text{CO}_2$  content may vary with the depth of respiration.

These two experiments illustrate also the fact that the final equilibrium in the bag is independent of slight changes in the diluting mixture. Thus, in Experiment 2 a lower  $\text{O}_2$  in the diluting mixture is associated with a higher  $\text{O}_2$  in the bag than in experiment 1.

#### COMMENTS

We have attempted to test our method, not only by the various means already described but also by comparing our results with known physiological facts. For example, the amount of oxygen taken up by the blood in the tonometers has with the exception of one out of twelve individuals been found to fall within the limits of oxygen dissociation curves published by Barcroft (1914) and others as normal.

The pH of the "mixed venous bloods," calculated from the relation of CO<sub>2</sub> tension and CO<sub>2</sub> content, is in agreement with the figures published by Peters, Barr and Rule for the pH of venous blood of normal resting subjects. We have laid particular stress upon the importance of an agreement between the relation of oxygen lost to CO<sub>2</sub> gained by a unit of blood and the respiratory quotient determined by the Tissot method. The respiratory quotient determined by the blood gases will agree with that determined by the Tissot method only when the figures for the oxygen and CO<sub>2</sub> contents of both arterial and venous blood are correct or else vary in the same direction and to the same extent from the correct figure. This agreement has been reasonably close in all our experiments in which some obvious error did not occur.

A consideration of our figures for the minute output of the heart is reserved for a separate paper. It may be stated here however that a comparison of blood flow figures would add very little as a check on the accuracy of any particular method, as there has been a striking lack of agreement in results obtained by different observers using different methods.

We have used standard methods of analyses and have therefore not described our analytical procedures. Our method for the most part is a combination of those that have been previously described. The technical contributions that we have made consist in the employment of alveolar air samples obtained by the Haldane-Priestly method after adjustment of the lung air, in order to determine the proper gas mixture to be used for diluting the lung air, the double rebreathing method for determining the correctness of the oxygen tension in the rebreathing bag, the method of obtaining a gas mixture in which all gases are in equilibrium with those of the venous blood, and the direct use of this gas mixture for obtaining so-called mixed venous blood by equilibration in a tonometer.

Many analyses of gas and blood are necessary to obtain all the data required for a complete experiment, but this burden falls on the workers and the procedures do not call for special training or endurance on the part of the subject. We believe therefore that our method is readily applicable to hospital patients of average intelligence who are not acutely ill.



## SUMMARY

A method for obtaining the gaseous content of the mixed venous blood of man has been developed that gives results for healthy resting subjects that fulfill practically all requirements of known physiological facts

When the results of the method are combined with those of analysis of the arterial blood and of the determination of the gas exchange in the lungs, data is at hand for calculating the output of the heart, per minute and per beat

The method is applicable to untrained subjects, and should be of value in the study of cardiac disease and of disturbances of the respiratory functions of the blood

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# THE RATE OF THE CIRCULATION OF THE BLOOD IN NORMAL RESTING INDIVIDUALS\*

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This paper describes two allied methods for the determination of the rate of blood flow which have yielded consistently reproducible results in untrained subjects. The data obtained in forty experiments on twenty-one normal resting individuals are presented in tabular form. The first method is suitable for use with subjects having practically any type of pathology but in the resting condition only. The second method is inapplicable when the subject has a pulmonary lesion preventing ventilation of part of the alveoli or a cardiac defect permitting mixture of arterial and venous blood. With these restrictions, however, this method should be useful as it can be carried out in a short time, with a high degree of accuracy and under varying conditions of activity.

## METHOD I

Several investigators have studied the circulation rate, determining the gases in the mixed venous blood by some procedure in which the lungs are used as an aerotonometer, and those in the arterial blood indirectly from the alveolar air or directly by analysis of blood obtained by arterial puncture. Burwell and Robinson, in a contemporary publication (1) have reviewed the literature of the subject and a complete bibliography will not be given here.

The method of Christianson, Douglas, and Haldane (2) is difficult to apply to patients but, in suitable subjects, should yield results of the correct order of magnitude. It consists in the inhalation of a mixture of  $\text{CO}_2$  and air (oxygen was used instead of air in a few ex-

\*The expenses of this research were defrayed in part by the Proctor Fund and by the Tutonal Fund of Harvard University

periments) of such proportions that two alveolar air samples taken at the end of half and complete expirations at five second intervals subsequent to the inhalation show no change or but slight change in  $\text{CO}_2$  content, indicating that they were in equilibrium with the  $\text{CO}_2$  of the mixed venous blood. This requires the use of several mixtures in order to obtain one that satisfies the above requirement or two that lie upon either side of the venous  $\text{CO}_2$  tension and sufficiently close to it. As the originators pointed out, the figure thus obtained represents the tension of  $\text{CO}_2$  in the mixed venous blood after it has become oxygenated without change in its  $\text{CO}_2$  content, and a correction must be applied in order to obtain the actual  $\text{CO}_2$  tension of the venous blood. This, they verified by experiments in which both the  $\text{CO}_2$  and  $\text{O}_2$  tensions were obtained simultaneously by "straddling" as described above. They determined the  $\text{CO}_2$  content of the arterial blood by applying the  $\text{CO}_2$  tension observed in the alveolar air by the Haldane-Priestly method (3) to the same  $\text{CO}_2$  dissociation curve to which the venous tension was applied. This curve did not need to be determined in each experiment for they assumed that, although the heights of different curves vary, the slopes are approximately the same.

Y. Henderson and Prince (4) pointed out that this method of obtaining the mixed venous  $\text{CO}_2$  tension was cumbersome and that the same result might be arrived at much more simply by a procedure in which expired air was inhaled from a rubber bag, held in the lungs for five seconds and exhaled into the bag. After this had been repeated several times the  $\text{CO}_2$  content of the mixture rose to a height at which it was constant through further repetitions. The figure thus obtained corresponded to  $\text{CO}_2$  tension of oxygenated mixed venous blood or, as Y. Henderson later (5) called it, the "virtual" venous  $\text{CO}_2$  tension.

Meakins and Davies (6) stated that they obtained indifferent results with the Henderson and Prince method of obtaining the mixed venous  $\text{CO}_2$  tension. This, they ascribed to improper mixing of the gasses in the lungs after a single inspiration and varied the procedure by having the subject exhale as deeply as possible, inhale from a bag of expired air, exhale deeply into the bag, inhale from the bag a second time, and finally exhale forcibly into the bag. A portion of the last of the air of the final expiration was removed into a Haldane gas samp-

ling tube through a side tube interposed between the mouthpiece and the bag. They also noted that, in patients, determinations of the arterial  $\text{CO}_2$  content by direct analysis of arterial blood were much more satisfactory than those made by interpolation of the alveolar  $\text{CO}_2$  tension on the  $\text{CO}_2$  dissociation curve.

Burwell and Robinson (1) have developed a method with which, by diluting the residual air with a proper mixture of  $\text{CO}_2$ ,  $\text{O}_2$ , and  $\text{N}_2$ , preliminary to the rebreathing, they obtain air in equilibrium with both the  $\text{O}_2$  and  $\text{CO}_2$  of a mixed venous blood. By analysis of the subject's blood equilibrated at body temperature in tonometers filled with gas from the rebreathing bag they obtain both the  $\text{CO}_2$  and  $\text{O}_2$  content of the mixed venous blood. Having analyzed directly both the  $\text{CO}_2$  and  $\text{O}_2$  of the arterial blood, they are able to determine the circulation rate from both gases and also the "respiratory quotient" of the blood. They thus have a double check on the accuracy of the procedure.

In our methods the  $\text{CO}_2$  only of the arterial and mixed venous bloods is determined. In the first method the  $\text{CO}_2$  content of arterial blood is determined both by direct analysis and by interpolation of the alveolar  $\text{CO}_2$  tension on the  $\text{CO}_2$  dissociation curve, determined in each experiment and corrected to 95 per cent oxygen saturation. The assumption that the arterial blood has an oxygen saturation of 95 per cent is, in all normal and in most pathological subjects, close enough to the actual condition so as to lead to no appreciable error. When the figures thus obtained by two different methods agree reasonably well there is afforded sufficient check as to the accuracy of the determinations. The case of disagreement will be discussed later.

The  $\text{CO}_2$  content of the mixed venous blood, we obtain by applying to the  $\text{CO}_2$  dissociation curve for completely oxygenated blood the  $\text{CO}_2$  tension obtained by the Henderson and Prince method modified in the following particulars. The equilibration time is prolonged to 15 seconds which is quite safe in the resting subject. The suggestion of Meakins and Davies (6) of rebreathing instead of holding the mixture in the lungs has been adopted but the subjects rebreathe four or five times instead of twice. This may be done in fifteen seconds without noticeable exertion. A mixture of 6 per cent  $\text{CO}_2$  with 94 per cent  $\text{O}_2$  rather than expired air is used for the rebreathing. Finally about

12 cc. of  $\text{CO}_2$  is generally added to the mixture between rebreathing periods in order to compensate for the diluting effect of the residual lung air and to raise the  $\text{CO}_2$  tension to that of the oxygenated mixed venous blood very quickly. This addition of  $\text{CO}_2$  between rebreathing periods is not essential in the case of resting subjects, at least, but it has given us more confidence in the equilibrations, especially in experiments with untrained subjects and in experiments during exercise when the more rapid blood flow necessitates reducing the period of rebreathing to five or ten seconds.

The use of a mixture with a high  $\text{O}_2$  content for the rebreathing seems essential if the assumption is to be made that the  $\text{CO}_2$  tension obtained is that of oxygenated venous blood. We have found  $\text{O}_2$  tensions of from 65 to 75 mm. of Hg in the gas mixtures after several rebreathing periods when the bag was filled in the beginning with expired air. Such tensions would be insufficient to oxygenate the blood completely even if complete equilibrium between it and the oxygen of the alveolar air were attained. But because of the slowness of  $\text{O}_2$  diffusion and because of inequalities of ventilation in different parts of the lungs together with the inability of the blood to equalize the  $\text{O}_2$  effects of such varying ventilation, as pointed out by Haldane (7), the  $\text{O}_2$  tension of blood leaving the lungs is considerably less than that of the alveolar air. The use of expired air for the rebreathing and the calculation of results on the assumption that complete oxygenation of the blood is thereby permitted causes an error that cannot readily be corrected because oxygenation will not be complete and the amount of unsaturation of the blood will not be known. By the use of high oxygen mixtures we have obtained figures for  $\text{CO}_2$  tensions from 0.5 to 1.0 mm. of Hg, higher than those obtained by rebreathing expired air. Table 1 shows the results of experiments in which bags containing the high oxygen mixture and expired air were used at alternating periods.

A mixture containing at the beginning 94 per cent of  $\text{O}_2$  may be used for five or six periods of rebreathing before the  $\text{O}_2$  tension falls below about 150 mm. of Hg. At this tension, with the increased depth of respiration used in the experiment, there will be no significant oxygen unsaturation of the blood passing through the lungs. As, under the conditions outlined above, the  $\text{CO}_2$  tension remains

TABLE 1

Experiment	CO <sub>2</sub> tensions in bags starting with	
	Expired air	High O <sub>2</sub> mixture
	mm. Hg	mm. Hg
1	41 2	42 2
	42 9	43 1
	41 9	43 2
	42 2	42 0
2	39 6	42 1
	39 8	42 1
	40 0	41 5
	39 0	39 0
3	46 65	46 8
	45 7	47 3
	45 3	46 2
	46 1	46 3

TABLE 2

Experiment	"Virtual" venous CO <sub>2</sub> tension	
	12 cc. CO <sub>2</sub> added between periods	No CO <sub>2</sub> added
	mm. Hg	mm. Hg
1	38 4	38 7
	40 1	39 5
	39 1	37 8
Average	39 2	38 7
2	40 6	41 7
	41 3	40 9
	40 7	41 5
Average	40 9	41 4
3	44 2	
	44 7	45 2
	44 8	44 7
	44 35	44 7
Average	44 5	44 9



practically constant from the second to the fifth or sixth rebreathing period, samples may be taken after each of four or five periods without renewing the gas mixture. This is ordinarily sufficient to give a satisfactory average.

For the validity of the assumptions that oxygenation of the venous blood, and  $\text{CO}_2$  equilibrium between it and the gas mixture rebreathed are complete, there are several bits of evidence. The fact that the addition of  $\text{CO}_2$  to the mixture between periods of rebreathing did not effect the  $\text{CO}_2$  tensions obtained is indication of the completeness of  $\text{CO}_2$  equilibrium. Table 2 shows the results of experiments in which two bags containing the same high oxygen mixture at the beginning were used alternately. To one of them 12 cc. of  $\text{CO}_2$  was added between periods. To the other there was no addition of  $\text{CO}_2$ .

The fact that variations in the  $\text{O}_2$  tension above 150 mm. of Hg have no effect on the  $\text{CO}_2$  tensions obtained is evidence of the completeness of the oxygenation of the blood even at the lower tensions of  $\text{O}_2$ . Table 3 shows the values of both  $\text{CO}_2$  and  $\text{O}_2$  in several series of determinations.

We endeavored to secure direct evidence by analysis of blood withdrawn from an artery during the last five seconds of a rebreathing period. Such blood should be completely oxygenated and should have the same  $\text{CO}_2$  tension as the air in the rebreathing bag. At the first attempt, made during the third period that the mixture was rebreathed, the blood was not obtained before the end of the fifteen second interval so that its  $\text{CO}_2$  content was increased by the second return of unventilated blood. It was, however, 99 per cent saturated with  $\text{O}_2$ —complete within the error of the analysis or near enough so as to have no appreciable effect on the  $\text{CO}_2$  tension.

At a second attempt sufficient blood was not obtained for both  $\text{O}_2$  and  $\text{CO}_2$  analyses. It appeared brightly arterial and the  $\text{CO}_2$  content, determined by direct analysis, was within 0.2 volume per cent of that estimated by interpolation of the  $\text{CO}_2$  tension of the mixture that was rebreathed on the  $\text{CO}_2$  dissociation curve for oxygenated blood subsequently determined. Table 4 is a protocol of this experiment.

The  $\text{CO}_2$  elimination was calculated from the  $\text{O}_2$  consumption determined with the Benedict portable metabolism apparatus, having our subjects in the post absorptive condition and assuming a respira-

tory quotient of 0.81. This seems to be by far the simplest and fully as accurate as any method except the use of the closed chamber

TABLE 3

Experiment	Gas mixture after rebreathing	
	O <sub>2</sub> tension	CO <sub>2</sub> tension
	<i>mm. Hg</i>	<i>mm. Hg</i>
1	347	46.6
	268	46.15
	246	46.8
	184	46.55
2	308	47.9
	271	47.8
	197	48.6
	151	48.2
3	284	42.8
	259	43.0
	192	43.0
	174	42.7
4	423	44.1
	343	44.7
	232	46.4
	158	44.7

TABLE 4

	CO <sub>2</sub> tensions	CO <sub>2</sub> content
	<i>mm. Hg</i>	<i>Vol. per cent</i>
"Virtual" venous blood (air after rebreathing)	47.3	
Arterial blood withdrawn from 15th to 20th seconds of re-breathing period		49.9
Points determined on CO <sub>2</sub> dissociation curve for oxygenated blood	44.5	49.1
	41.7	47.7
	44.75	48.8
Arterial blood (by interpolation on dissociation curve)	46.8	
Height of dissociation curve at 47.3 mm. of Hg tension		50.1

Variations in the respiration while the subject is on the apparatus can influence the oxygen consumption but little, while the CO<sub>2</sub> elimination and the respiratory quotient will be very seriously effected

Consideration of table 5 will convince one of this latter statement. The first six columns are copied from the paper of Hendry, Carpenter and Emmes (8). Each figure represents the average respiratory quotient in two of twelve periods done on each subject in a morning.

The average respiratory quotient for the seventeen subjects is 0.814. In three of the subjects the values obtained for the different periods varied so greatly and some were so far from the usual post-absorptive value that not even the average of twelve periods may be assumed to represent the actual quotients. Excluding these three subjects, J. A. C., H. O., and P. G. H., the average quotient of the remaining fourteen is 0.809. Without much doubt an assumed respiratory quotient of 0.81 would have approached the actual conditions in the three subjects named more closely than did the average of twelve determinations.

Also, it will be apparent that even in the remaining fourteen subjects it would have required considerably more than two periods to determine a respiratory quotient closer to the actual than an assumed value of 0.81. In none of these fourteen did the actual quotient, if that is assumed to be represented by the average of the twelve periods, differ from 0.81 by more than 0.023 and the average deviation from 0.81 was only 0.014. On the other hand in eleven of the fourteen there was a maximum deviation of the average of two periods from the average of the twelve periods of more than 0.02 and for the fourteen the average maximum deviation was 0.032. The average deviation of the average of two periods from that for the individual was 0.018 throughout the fourteen. This occurred despite the fact that the determinations were done in a laboratory where every possible care and precaution to eliminate errors were taken. They represent a degree of accuracy that will not be readily duplicated.

The order in which the various specimens and data are collected in our procedure is of some importance. The experiment must be so arranged as to consume a minimum amount of time as it is found that if it is too prolonged the level of respiratory control may change so that both the alveolar and "virtual" venous  $\text{CO}_2$  tensions become either greater or less than at the beginning of the experiment or at the time when the sample of arterial blood is withdrawn. Experiments Nos. 1 and 2 of table 2 illustrate this change in level. The metabo-

TABLE 5

Subject	Date	Periods of experiment						Average	Variation from 0.81	Maximum variation between averages of two periods	Maximum deviation of two periods from average of individual	Average deviation of two periods from average of individual
		1 and 2	3 and 4	5 and 6	7 and 8	9 and 10	11 and 12					
J A C	April 8	0.76	0.79	0.83	0.77	0.72	0.70	0.762	0.048	0.13	0.068	0.035
C A C	April 9	0.81	0.81	0.79	0.79	0.78	0.77	0.792	0.018	0.04	0.022	0.012
II II II	April 10	0.84	0.85	0.83	0.82	0.82	0.83	0.832	0.022	0.03	0.018	0.009
I II N	April 11	0.79	0.80	0.80	0.79	0.81	0.81	0.80	0.010	0.02	0.010	0.007
J I T	April 12	0.86	0.85	0.83	0.82	0.80	0.80	0.826	0.016	0.06	0.034	0.020
A G N	April 15	0.78	0.77	0.86	0.85	0.77	0.87	0.817	0.007	0.10	0.053	0.043
J L G	April 16	0.87	0.78	0.84	0.81	0.78	0.80	0.813	0.003	0.09	0.057	0.023
I S	April 17	0.83	0.82	0.78	0.81	0.77	0.78	0.798	0.012	0.06	0.032	0.022
W I M	April 18	0.82	0.81	0.85	0.81	0.84	0.84	0.828	0.018	0.04	0.022	0.015
S N G	April 23	0.76	0.79	0.79	0.76	0.81	0.82	0.788	0.022	0.06	0.032	0.019
W J S	April 25	0.77	0.77	0.79	0.76	0.84	0.83	0.793	0.017	0.08	0.047	0.028
C S B	April 26	0.78	0.79	0.79	0.82	0.75	0.79	0.787	0.023	0.07	0.037	0.011
II O	April 30	0.97	0.76	0.86	1.03	0.67	0.83	0.853	0.013	0.36	0.183	0.100
I G II	May 1	0.84	0.95	0.92	0.86	0.88	0.91	0.893	0.083	0.11	0.057	0.035
C I M	May 2	0.83	0.82	0.84	0.82	0.79	0.84	0.823	0.013	0.05	0.033	0.013
R K B	May 3	0.80	0.80	0.81	0.77	0.81	0.80	0.798	0.012	0.03	0.028	0.010
II B	May 6	0.85	0.82	0.82	0.85	0.83	0.82	0.832	0.022	0.03	0.018	0.012
Average		0.82	0.81	0.83	0.82	0.79	0.81	0.814	0.023	0.08	0.044	
Average, excluding J A C, II O, and I G II												0.018
										0.05	0.032	

lism is the last thing to be done as it is found that the abnormality of ventilation which is very apt to occur while the subject is connected with the apparatus is accompanied by more rapid readjustment of base in the blood than had been thought possible

After a rest period of from 45 to 60 minutes, one or two alveolar air samples are collected. The first of five 15 second periods of re-breathing from the bag to obtain the oxygenated venous  $\text{CO}_2$  tension is then begun. Samples are removed from the bag after each of the last four of these periods. If more samples are desired the gas mixture in the bag must be renewed. An alveolar air sample is collected one minute before and again three minutes after each rebreathing period. Between periods 2 and 3 the radial artery is anesthetized with novocaine and between periods 3 and 4 the sample of arterial blood is withdrawn. This entire procedure may usually be completed within about thirty minutes.

It is not necessary to discuss the details of our technique as they have been described in previous papers (9, 10). Suffice it to say, largely in recapitulation, that the  $\text{CO}_2$  dissociation curve for oxygenated blood was determined in each experiment. Sufficient arterial blood was usually obtained for this purpose. When such was not the case and the supply was supplemented by venous blood drawn without stasis, no significant difference between arterial and venous blood was noted. The alveolar and "virtual" venous  $\text{CO}_2$  tensions and the  $\text{CO}_2$  content of arterial blood were determined by direct analysis. The  $\text{CO}_2$  content of the mixed venous blood and the  $\text{CO}_2$  tension of the arterial blood were estimated by interpolation of the corresponding  $\text{CO}_2$  tension and content on the  $\text{CO}_2$  dissociation curves for blood completely oxygenated and 95 per cent oxygenated, respectively.

Only the collection of alveolar air samples requires further comment. We have recently presented theoretical and experimental evidence (11) indicating that the effective alveolar  $\text{CO}_2$  tension is very closely approximated by that of the Haldane-Priestly sample collected at the end of normal expiration rather than by the average of samples collected at the end of both expiration and inspiration. We believe that the latter procedure yields results that are 1 to 2 mm. of Hg tension too low. Consequently only expiratory alveolar air samples have been taken.

## METHOD II

In experiments done according to the method described above there have been discrepancies between the arterial and the alveolar  $\text{CO}_2$  tensions. In most instances these discrepancies have been small and that fact is indicative of the accuracy of the determinations, it making little difference, in the majority of cases, whether the circulation rate is calculated using the arterial  $\text{CO}_2$  content directly determined or that estimated from the alveolar  $\text{CO}_2$  tension. In a few experiments, however, the discrepancies have been large. In such a case it is difficult to know which is the correct value to use.

Discrepancies between the alveolar and arterial  $\text{CO}_2$  tensions of even greater magnitude than we have found have been observed by previous workers (12, 13). They have usually been ascribed to incompleteness of  $\text{CO}_2$  equilibrium between alveolar air and arterial blood but, as has been shown by Van Slyke (14), this can hardly be the correct explanation.  $\text{CO}_2$  diffusion being 20 to 30 times as rapid as  $\text{O}_2$  diffusion there can never be any considerable difference between alveolar and arterial  $\text{CO}_2$  tensions while there is adequate oxygenation of the arterial blood. The difference observed can be readily explained as the effect of variations in ventilation between the times of collection of the samples of alveolar air and arterial blood. Inasmuch as the  $\text{CO}_2$  tension of the alveolar air is inversely proportional to the pulmonary ventilation, the  $\text{CO}_2$  elimination remaining constant, it is apparent that the respirations need vary but little to change this tension considerably.

Believing this to be the case, we have devised the second method in order to avoid the error due to such variations in alveolar  $\text{CO}_2$  tension and to provide a more rapid means of estimating the rate of blood flow. The object of this method is to obtain simultaneous values of the alveolar and "virtual"  $\text{CO}_2$  tensions. This we have been able to do by means of a simple piece of apparatus using a three way and a two way valve (Fig. 1). The three way valve (*a*) to which a mouth piece is attached permits the subject to breathe either through the outlet (*b*) into the outside air or through the rest of the apparatus. The two way valve (*c*) may be turned either into a long tube (*d*) which has a side tube (*e*) close to the valve for collection

of alveolar air samples or into a rubber bag (*f*) containing the  $\text{CO}_2$  and  $\text{O}_2$  mixture for equilibration with oxygenated venous blood

During the experiment the subject has the mouthpiece in his mouth with the three way valve (*a*) turned so that he is breathing into the outside air. The two way valve (*c*) is turned into the tube (*d*). At the end of a normal expiration the valve (*a*) is turned so that the subject may make a forced expiration through the valve (*c*) and the tube (*d*). At the end of the forced expiration the valve (*c*) is turned so that the subject may breathe from the bag (*f*). He rebreathes the gas mixture in the bag four or five times and then near the end of the 15 second period exhales deeply into the bag. At the end of this

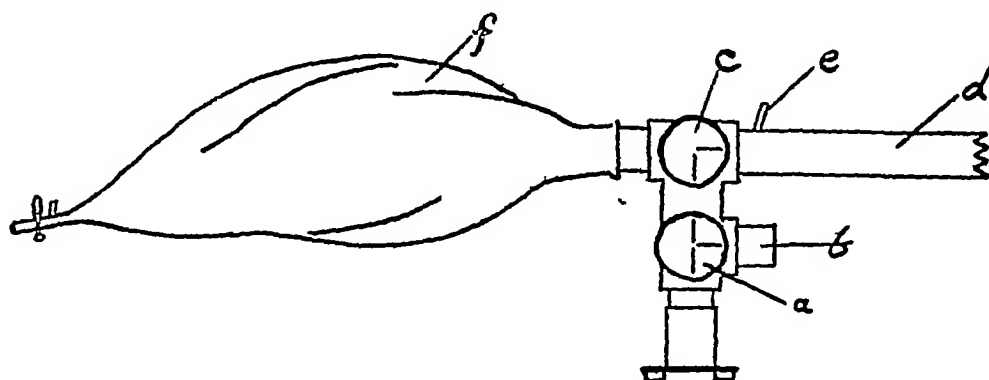


FIG 1 APPARATUS FOR COLLECTION OF ALVEOLAR AND "VIRTUAL" VENOUS AIR SAMPLES

expiration the valve (*a*) is again turned so that the outside air is breathed. A sample of alveolar air is taken into a Haldane sampling tube from the side tube (*e*), (this may be done during the rebreathing from the bag, the sampling tube being previously attached to the apparatus) and a sample of the air in the bag is taken. Between periods about 12 cc of  $\text{CO}_2$  are usually introduced into the bag.

In this way there is obtained from the bag air that is in  $\text{CO}_2$  equilibrium with the oxygenated venous blood that was in the peripheral circulation at the time when the alveolar air sample was taken. The two samples thus yield simultaneous values. The circulation rate remaining the same, variations in pulmonary ventilation should be reflected in both the arterial and venous  $\text{CO}_2$  tensions and the difference between the two should not be effected thereby. This is true, however, only

within certain limits. The tissues serving as a reservoir for  $\text{CO}_2$ , the venous  $\text{CO}_2$  tension follows changes in the arterial  $\text{CO}_2$  tension comparatively slowly. Hence if there has been a marked change in ventilation immediately preceding the collection of the samples an incorrect value for the difference between the arterial and venous  $\text{CO}_2$  tensions will be obtained. In order to reduce the probability of this error we have had our subjects keep the mouthpiece in place throughout the time that the samples were being collected. The biggest change in ventilation occurs when the subject shifts from normal nose breathing to breathing through the mouthpiece of the apparatus. Once the breathing has become steady at the new level and there has been time for adjustment of the  $\text{CO}_2$  tension of the tissues to it, the change has no effect on the difference between arterial and venous

TABLE 6

Tension range	Factor for slope of $\text{CO}_2$ dissociation curve
<i>mm Hg</i>	
45 to 50	0.42
40 to 50	0.44
40 to 45	0.46
35 to 40	0.48
30 to 40	0.51

$\text{CO}_2$  tensions. Minor differences in ventilation are followed by rapid enough adjustment so that values of a good degree of uniformity may usually be obtained and the average of several of these values should give a very accurate figure.

The calculation of the circulation rate from the figures so obtained is simple. One millimeter is subtracted from the average difference in  $\text{CO}_2$  tension between the alveolar air and the oxygenated venous blood. This is because of the small difference in  $\text{CO}_2$  tension that probably exists between the alveolar air and arterial blood for reasons discussed in another paper (11) and because arterial blood is only about 95 per cent saturated with oxygen while the high oxygen mixture used results in practically complete oxygen saturation of the blood passing through the lungs during the rebreathing. This figure, of course, is only an estimate but it is probably nearly correct and an error of a few tenths of a millimeter would make but little difference



in the final result. The corrected figure for tension difference is then applied to a standard  $\text{CO}_2$  dissociation curve for oxygenated blood to reduce it to terms of cubic centimeters. This may be most readily done by multiplying by a factor corresponding to the slope of the dissociation curve. We have not assumed a straight line dissociation curve although such an assumption would not cause a very great error. We have used a set of factors corresponding to the different ranges of tension over the  $\text{CO}_2$  dissociation curve of A. V. B., published elsewhere (10). Table 6 contains these factors. They are accurate enough for use with all subjects whose blood contains normal amounts of base and hemoglobin. In other subjects the slope of the curve must be determined otherwise. The data in hand then permit the application of Fick's principle by which the figure for the blood flow is obtained.

#### DISCUSSION

Forty determinations of the circulation rate have been made on twenty-one individuals, all of them young adults, three of them females. The protocols of these experiments are contained in Tables 7 and 8. In the instances where repeated determinations have been made on the same individual they usually agree reasonably well. In the majority of them the variation is a fraction of a liter. In a few cases it is several liters. Examination of the data of such experiments leads one to conclude that the variations are in large part real. Indeed, they are to be expected for it is probable that the circulation rate is influenced by many factors that are beyond control of our attempts to secure basal conditions. One would expect, for instance, that the circulation rate would be more variable than the basal metabolic rate.

When the repetitions have been made by the different methods the agreement is again good. In general the figures obtained by the second method are somewhat lower than those by the original one. Of the two we consider that the second method is somewhat less liable to error because of the simultaneous nature of the determinations of alveolar and venous  $\text{CO}_2$  tensions.

Concerning the normal circulation rate under basal conditions the data herein reported would indicate that the contenders for a

TABLE 7

Date	Subject	Height cm	Weight kilos	Pulse per minute	Arterial CO <sub>2</sub> content vol per cent	Height of CO <sub>2</sub> dissociation curve at tension of 40 mm. of Hg vol per cent	Arterial CO <sub>2</sub> tension mm Hg	Alveolar CO <sub>2</sub> tension mm Hg	Arterial CO <sub>2</sub> content es- timated from alveolar CO <sub>2</sub> tension vol per cent	"Virtual" venous CO <sub>2</sub> tension mm Hg	Venous CO <sub>2</sub> content vol per cent	Actual CO <sub>2</sub> tension of venous blood mm Hg	Oxygen capacity vol per cent	Oxygen saturation of venous blood per cent	CO <sub>2</sub> elimination per min liters	Circulation rate per min liters	Circulation rate of alve- olar CO <sub>2</sub> tension estimated on basis of alve- olar CO <sub>2</sub> tension liters	Circulation per kilo per beat
3-22-24	I W L	179.5	72.0	50	50.4	49.1	11.6	10.7	49.25	18.21	53.45	45.2	20.0	77.0	198.4	6.61	7.16	1.82
4-4-24	I W I	179.5	72.0	48-53	49.2	18.4	11.1	10.7	51.6	50.05	51.8	46.4	19.9	76.6	204.0	7.03	6.37	1.95
4-9-24	I W I	179.5	72.0	49	51.75	50.6	41.75	11.0	47.3	15.6	50.2	41.9	18.2	74.0	204.0	6.69	4.81	1.90
4-10-24	I T II	171.5	51.6	51	47.1	47.7	38.0	38.6	47.8	45.3	50.2	42.8	21.5	83.0	139.3	4.5	7.14	1.52
4-18-24	C M J	166.0	65.4	64	48.0	47.8	39.6	39.9	48.15	16.5	50.6	44.3	20.3	83.2	155.0	7.80	6.33	1.85
5-2-24	C M J	166.0	65.4	61	48.57	47.6	41.4	39.9	48.15	16.5	50.6	44.3	20.3	83.2	155.0	7.80	6.33	1.91
5-2-24	J M I	185.0	68.6	56	50.6	50.2	10.1	35.15	48.6	47.0	53.3	13.7	18.0	77.2	201.7	7.17	1.29	1.91
4-11-24	A V B	175.0	68.0	60	45.7	46.2	37.1	37.1	46.4	15.2	19.7	41.2	20.0	70.8	190.3	4.76	5.77	1.17
4-25-24	A V B	175.0	68.0	66	45.1	46.2	37.1	37.1	46.4	15.2	19.7	41.2	20.0	70.8	190.3	4.76	5.77	1.17
3-28-24	II I	171.0	66.1	77	46.55	47.5	37.5	37.13	46.6	41.2	18.1	39.5	20.1	85.4	200.0	12.9	13.2	1.06
4-7-24	II I	171.0	66.1	72	46.15	46.8	38.6	37.2	46.0	13.3	18.45	41.2	20.8	83.6	208.0	10.1	8.5	2.19
4-15-24	II I	171.0	66.1	71	46.6	46.95	38.7	37.4	46.0	43.0	48.3	41.2	20.8	83.6	208.0	10.1	8.5	2.18
4-17-24	II I	171.0	66.1	71-76-78	46.11	46.95	35.2	35.6	46.0	10.5	47.2	38.2	22.1	82.6	193.8	11.75	8.7	2.39
5-3-24	II I	171.0	66.4	71	45.6	47.2	36.0	36.5	46.1	10.6	47.5	38.5	21.0	84.3	213.0	11.2	11.5	1.67
4-22-24	S L W	192.3	85.5	62	49.0	50.7	36.0	39.5	51.0	45.05	53.0	42.1	22.6	74.0	214.0	5.35	10.6	2.38
4-23-24	II P S	176.5	82.7	65	46.6	41.75	43.15	42.9	46.7	19.3	48.95	16.6	21.7	82.1	212.0	9.03	9.12	1.75

TABLE 8

Date	Subject	Sex	Height	Weight	Pulse per minute	Pair of sam- ples	CO <sub>2</sub> tension				Sam- ples aver- aged	Difference arterial and venous CO <sub>2</sub> content	CO <sub>2</sub> elimina- tion per minute	Circula- tion rate per minute	Output per kilo per beat
							Alveolar	"virtual" venous	Differ- ence	Average difference					
			cm	kilos	per minute	number	mm Hg	mm Hg	mm Hg	mm Hg	number	vol. per cent	cc	liters	cc
1924 5-23	E F G	M	186	91	58	1	40 1	46 3	6 2	6 73	1	2.58	238	9 23	1 75
						2	40 1	47 3	7 2		2				
						3	38 8	45 7	6 9		3				
						4	39 85	46 55	6 7		4				
						5	41 1	47 75	6 65		5				
5-24	H F	M	174	66	74	1	35 55	41 0	5 45	5 4	1	2 11	207	9 81	2 01
						2	33 6	38 7	5 1		2				
						3	33 4	39 4	6 0		3				
						4	36 4	41 8	5 4		4				
						5	37 9	42 9	5 0		5				
7-18	H F	M	174	65	74	1	38 6	43 4	4 8	5 2	1	1 93	193	10 0	2 08
						2	37 9	43 75	5 85		2				
						3	39 5	44 8	5 3		3				
						4	39 5	44 6	5 1		4				
						5	39 6	44 6	5 0		5				
6-10	H F	M	174	66	72	1	37 95	43 2	5 25	6 03	1	2 36	191	8 1	1 70
						2	37 4	43 95	6 55		2				
						3	37 05	43 7	6 65		3				
						4	37 2	42 4	5 2		4				
						5	37 5	42 1	4 6						
						6	38 75	43 7	5 95		6				
						7	37 9	44 5	6 6		7				

9-6	H T	M	174	64	67	1	39 2	43 7	4 5	5 7	2 16	198 5	9 19	2 14
						2	39 15	44 6	5 45					
						3	39 0	44 85	5 85					
						4	38 9	45 1	6 2					
						5	39 1	44 3	5 2					
5-27	F W L	M	179 5	72	52	1	39 45	47 15	7 7	8 55	3 47	204 5	5 89	1 57
						2	40 4	45 3	1 9					
						3	38 8	47 7	8 9					
						4	34 45	45 75	11 3					
						5	36 1	44 7	8 6					
						6	36 65	15 65	9 0					
5-28	J M T	M	185	68 6	48	1	37 7	44 1	6 4	7 3	2 90	199 2	6 87	2 09
						2	38 3	45 3	7 0					
						3	38 4	15 9	7 5					
						4	38 85	46 3	7 15					
6-4	G C R	M	165 5	87 5	64	1	36 6	48 3	11 7	10 49	4 18	196 7	4 7	0 84
						2	40 9	50 15	9 25					
						3	39 95	49 6	9 65					
						4	40 15	46 65	6 5					
						5	39 3	18 2	8 9					
						6	40 0	50 75	10 75					
						7	38 95	50 2	11 25					
						8	40 8	51 15	10 35					
6-5	C M J	M	166	65 1	62	1	43 4	50 3	6 9	7 43	2 77	179	6 18	1 60
						2	42 75	50 5	7 75					
						3	43 7	51 9	8 2					
						4	42 6	19 6	7 0					
						5	42 95	50 2	7 25					
						6	43 1	50 6	7 5					

TABLE 8—Continued

Date	Subject	Sex	Height cm	Weight kilos	Pulse per minute	Pair of sam- ples number	CO <sub>2</sub> tension				Sam- ples aver- aged number	Difference arterial and venous CO <sub>2</sub> content vol. percent	CO <sub>2</sub> elimina- tion per minute cc	Circula- tion rate per minute liters	Output per kilo per beat cc
							Alveolar mm Hg	"Virtual" venous mm Hg	Differ- ence mm Hg	Average difference mm Hg					
1924 6-10	H N S	M	164	67	60	1	42.3	49.8	7.5	8.8	1	3.43	194.3	5.66	1.41
						2	40.1	48.7	8.6		2				
						3	40.2	49.2	9.0		3				
						4	40.9	47.6	6.7		5				
						5	40.95	49.65	8.7		6				
						6	41.7	50.0	8.3						
6-13	H N S	M	164	67	65	1	39.1	47.8	8.7	8.4	1	3.26	200.5	6.15	1.41
						2	42.3	49.1	6.8						
						3	41.4	49.6	8.2		3				
						4	41.4	49.8	8.4		4				
6-11	A K	M	166	69.1	61	1	41.55	45.8	4.25	7.11	2	2.75	182.3	6.63	1.57
						2	41.9	48.45	6.55		3				
						3	41.7	48.6	6.9		4				
						4	41.25	48.5	7.25		5				
						5	40.7	48.45	7.75						
						6	39.7	48.6	8.9						
6-12	W L McK	M	185	88.7	64	1	41.75	49.8	8.05	7.83	1	3.05	205.5	6.72	1.18
						2	42.3	50.75	8.45		2				
						3	42.75	50.65	7.9		3				
						4	42.3	48.05	5.75						
						5	42.7	49.9	7.2		5				
						6	42.85	50.4	7.55		6				

6-13	A C R	M	178	77	54	1	32 3	43 5	11 3	8 8		3 74	201	5 38
						2	30 75	40 85	10 1					
						3	30 7	39 9	9 2		3			
						4	28 65	37 45	8 8		4			
						5	29 2	37 8	8 6		5			
						6	26 5	35 1	8 6		6			
6-11	W B C	M	190 5	81 8	59	1	40 8	18 8	8 0	7 81	1	3 06	223	7 28 1 51
						2	42 2	50 1	7 9		2			
						3	38 5	46 75	8 25		3			
						4	40 05	47 85	7 8		1			
						5	13 8	49 7	5 8					
						6	13 4	50 5	7 1		6			
6-19	A V B	M	175	68	66	1	11 6	18 5	6 9	7 85		3 08	190 3	6 18 1 38
						2	11 8	19 9	8 1		2			
						3	40 55	18 7	8 15		3			
						1	41 95	19 25	7 3		4			
9-3	A V B	M	175	68	64	1	38 5	19 7	11 2	9 91	1	1 02	180 5	1 19 1 03
						2	10 2	50 0	9 8		2			
						3	39 6	18 7	9 1		3			
						4	36 4	16 4	10 0		1			
						5	37 1	17 0	9 6		5			
7-1	P D	M	191	70	63	1	13 6	50 8	7 2	7 1	1	2 62	213 0	8 13 1 84
						2	41 65	50 45	5 8					
						3	13 65	50 55	6 9		3			
						4	12 6	19 85	7 25		4			
7-3	P D	M	191	69 9	60	1	13 35	50 9	7 55	7 1	1	2 62	196 0	7 17 1 78
						2	12 65	50 55	7 9		2			
						3	11 1	50 6	6 5		3			
						1	14 25	50 6	6 15		4			

TABLE 8—Concluded

Date	Subject	Sex	Height	Weight	Pulse	Pair of sam- ples	CO <sub>2</sub> tension				Sam- ples aver- aged	Difference arterial and venous CO <sub>2</sub> content	CO <sub>2</sub> elimina- tion per minute	Circula- tion rate per minute	Output per kilo per beat
							Alveolar	"Virtual" venous	Differ- ence	Average difference					
1924			cm	kilos	per minute	number	mm Hg	mm Hg	mm Hg	mm Hg	number	vol. per cent	cc.	liters	cc.
7-2	A M B	M	173	62 0	65	1	33 8	39 85	6 05	5 5	1	2 16	180	8 34	1 97
						2	34 6	39 9	5 3	2					
						3	35 7	40 7	5 0	3					
						4	34 9	40 55	5 65	4					
7-7	J C E	M	176	66 5	50	1	47 3	54 5	7 2	6 8	1	2 44	207 6	8 51	2 56
						2	48 45	54 5	6 05	2					
						3	47 55	54 65	7 1	3					
						4	49 65	54 75	5 1						
6-11	M E M	F	166	56	60	1	40 25	46 75	6 5	7 99		3 08	194 7	5 13	1 53
						2	40 75	49 4	8 65	2					
						3	41 55	49 35	7 8	3					
						4	40 55	48 9	8 35	4					
						5	40 35	47 5	7 1	5					
6-16	M F H	F	160	51 4	70	1	38 5	44 5	6 0	6 26	1	2 45	162	6 63	1 84
						2	37 2	44 3	7 1	2					
						3	38 2	42 5	4 3						
						4	37 75	44 0	6 25	4					
						5	37 3	44 0	6 7	5					
7-19	M A S	F	162 5	52	62	1	41 55	46 55	5 0	5 8	1	2 16	161	7 46	2 31
						2	41 65	47 35	5 7	2					
						3	41 3	47 55	6 25	3					
						4	39 9	46 75	6 85	4					
						5	37 5	42 7	5 2	5					

high and a low resting blood flow are both right. The series is large enough so that several individuals of both types have been encountered, as well as variations between the two extremes. The majority of the determinations have fallen within Y. Henderson's estimate (15) of a cardiac output of 1.5 to 2.0 cc per kilo per beat. Five subjects have had one or more determinations that were below these figures and four have had larger outputs.

In at least four individuals, the small cardiac output under conditions of rest would appear to render impossible Y. Henderson's conclusion (15) that the stroke volume of the heart is a nearly constant quantity for each individual, it being too small to permit sufficient ventilation of the tissues during strenuous exercise. In the others, a constant stroke volume would be possible.

No correlation between the volume of the blood flow and any other factor, such as type of build or athletic ability is apparent.

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# THE GASEOUS CONTENT OF THE BLOOD AND THE OUTPUT OF THE HEART IN NORMAL RESTING ADULTS

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## I GASEOUS CONTENT OF THE BLOOD

In a previous paper (Burwell and Robinson, 1924) we have described a respiratory procedure by which a gas mixture is obtained in which both oxygen and carbon dioxide are in equilibrium with those gases in the blood entering the lungs (the mixed venous blood). The equilibration of this gas with blood of the subject allows, therefore, the determination of the oxygen and carbon dioxide content of the mixed venous blood. We used the analytical method of Van Slyke and Stadie. The data thus obtained, together with those obtained by measuring the gas exchange in the lungs, and by counting the pulse, allow the output of the heart per minute and per beat, by a method to be described in the second part of this paper.

*Material* In the present paper we wish to report the results of a study of eleven normal resting subjects, all of whom are members of a hospital staff, ranging in age from twenty-five to forty-five years. All observations were made in the morning, after about half an hour's rest in a reclining chair, and at least twelve hours after taking food. As nearly identical conditions as possible were obtained in all determinations.

*Gaseous tension of the mixed venous blood* In table 1 are brought together the results of a number of observers. In all instances the principle of using the lungs as an aerotonometer, as suggested by Pflüger, has been employed. Loewy and v. Schrotter (1905) measured the tension of oxygen and carbon dioxide in gas withdrawn from an occluded portion of a lung by means of a lung catheter. All other observers have used some modification of the rebreathing method introduced by Plesch (1909).

The figures of Loewy and v Schrotter are the average of a number of determinations with fairly wide variations, while the figures of others are either determinations on one subject or show the limits found in small series. Our own findings show a somewhat greater

TABLE 1  
*Oxygen and carbon dioxide tension of the mixed venous blood*

Authors	Oxygen tension	CO <sub>2</sub> tension
	mm Hg	mm Hg
Loewy and v Schrotter (1905)	37 5	42 5
Fridencia (1918)	35 1-44 5	45 2-46 3
Barcroft, Roughton and Shoji (1922)	32 6	49 5
Douglas and Haldane (1922)	46 1	44 0
Meakins and Davies (1922)		44 4-49 1
Redfield, Bock and Meakins (1922)	31 0-36 0	45 0-50 0
Burvell and Robinson	31 6-43 7	41 0-47 0

TABLE 2  
*Oxygen and carbon dioxide content of the blood*

Subject	Arterial blood		Mixed venous blood		Oxygen utilized		Respiratory quotient		pH of mixed venous blood
	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>		Of total oxygen capacity	From blood gases	Gas exchange	
	vol per cent	vol per cent	vol per cent	vol per cent	vol per cent	per cent			
1 A	21 76	46 01	15 23	50 70	6 53	31	0 72	0 76	7 34
2 R	22 23	44 75	16 26	49 61	5 97	25	0 81	0 85	7 33
3 H	22 60	45 02	17 01	49 52	5 59	23	0 80	0 81	7 32
4 F	21 80		16 10		5 70	24		0 72	
5 B	23 04	46 43	18 15	49 80	4 89	21	0 69	0 71	
6 L	21 80	47 36	16 55	51 18	5 25	23	0 73	0 75	7 34
7 G ♀	17 36	46 26	13 08	49 52	4 28	24	0 76	0 82	7 34
8 M	22 59		17 14		5 45	23		0 76	
9 P	21 11	50 30	15 95	54 00	5 16	24	0 72	0 78	7 35
10 K	21 30		17 21		4 09	18		0 71	
11 Bu	21 00	47 29	17 86	49 63	3 14	14	0 75	0 75	7 33

variation than previously reported, but our figures are of the same order as those obtained by others. Our own figures, of course, are individual determinations.

*The gaseous content of the blood* The oxygen and carbon dioxide content of the blood was determined by the method described in the

previous paper Table 2 shows the results of these analyses in the eleven subjects The oxygen utilized in the passage of the blood through the body is shown in cubic centimeters utilized from each 100 cc of blood and also as the percentage of the total oxygen capacity of the blood The respiratory quotient has been calculated from the blood gases by dividing the amount of the  $\text{CO}_2$  lost by 100 cc of blood by the amount of oxygen gained by 100 cc. of blood in passing through the lungs The figures so obtained may be compared with the respiratory quotient calculated from the gas exchange in the lungs, which was determined by the Tissot method during each experiment. The fairly close agreement that exists may be taken as evidence of the accuracy of the blood gas determinations A rational respiratory quotient is obtained only when the four determinations for arterial and venous oxygen and carbon dioxide are substantially correct.

The percentage saturation of the blood exposed to the "venous gas mixture" has in general fallen within the limits of previously published dissociation curves Sufficient variation exists, however, to render the use of so-called standard dissociation curves unsatisfactory

The pH figures calculated from the  $\text{CO}_2$  tension and content agree with those of others for normal subjects, although they are slightly lower than the average determinations of several recent observers This agreement indicates that no outspoken error occurred in the  $\text{CO}_2$  analyses of the venous blood, and that as a rule no pronounced acid change occurred during the handling of the blood

## II THE OUT-PUT OF THE HEART

During the past hundred years the problem of the actual volume of the blood expelled by the heart has been attacked frequently by both speculation and experiment The shrewd guess of Thomas Young (1808) put the output of the heart per beat at an ounce and a half Such estimations, which were based chiefly upon postmortem measurement of the capacity of the ventricles, were followed by experimental observation upon animals and application of the data so obtained to the calculation of the output of the heart in man These calculations have resulted in figures so confused and variable that it is quite clear that acceptable determinations must be made upon human subjects

Many workers have made observations upon human subjects and various types of data have been the bases of the calculation of the volume of the circulation per minute and per beat in man (1) the volume flow in the arm, determined by plethysmographic methods (Muller, 1909), (2) the measurements of instantaneous x-ray photographs of the heart in systole and diastole (Meek and Eyster, 1923), (3) the amount of some inert gas taken up by the blood in a given time, when the absorption coefficient of the gas for blood is known (Bornstein, 1910, Krogh and Lindhard, (1912), (4) the application of the principle of Fick (Fick, 1870, Loewy u v Schrotter, 1905, Plesch, 1909, Douglas and Haldane, 1922) Of these the last two types of data are at present the most acceptable

It would serve no useful purpose to present again the diverse figures for the circulatory minute volume that have been obtained by different workers using different methods Not only were the methods widely different but also the conditions of the experiments, the activity, position, and external temperature of the subjects were so varied that the results are in no strict sense comparable It is worthwhile, however, to point out certain general tendencies in these widely divergent figures The recorded minute volume varies from 2,800 cc to 9,000 cc per minute for normal resting adults There are roughly speaking two groups, in the first are subjects with minute volumes of from 3,000 to 5,000 cc, having usually an output per beat of 45 to 75 cc In the second group are those with minute volumes of 6,500 to 8,500 cc, having an output per beat of 100 cc or more The existence of these two groups of figures has been partly responsible for the development of two contrasting beliefs as to the method of response of the circulation to demand for increased blood supply, a question that will be discussed in a subsequent paper dealing with the response of the circulation to exercise

The measurements of the output of the heart which we wish to report have been obtained by the application of a method based upon Fick's principle, a principle best described in the words of its originator

Man bestimme, wie viel Sauerstoff ein Thier während einer gewissen Zeit aus der Luft aufnimmt und wie viel Kohlensäure es abgibt Man nehme ferner dem Thiere während der Versuchszeit eine Probe arteriellen und eine Probe venösen Blutes In Beiden ist der Sauerstoffgehalt und der Kohlen-

säuregehalt zu ermitteln Die Differenz des Sauerstoffgehaltes ergibt, wie viel Sauerstoff jedes Cubiccentimeter Blut beim Durchgang durch die Lungen aufnimmt, und da man weiss, wie viel Sauerstoff im Ganzen während einer bestimmten Zeit aufgenommen wurde, so kann man berechnen, wie viel Cubiccentimeter Blut während dieser Zeit die Lungen passirten, oder wenn man durch die Anzahl der Herzschläge in dieser Zeit dividirt, wie Cubiccentimeter Blut mit jeder Systole des Herzens aufgeworfen wurde Die entsprechende Rechnung mit den Kohlensäuremengen gibt Bestimmung desselben Werthes, welche die erstere controllirt

As the gases of the blood are now commonly calculated as volumes of gas per 100 cc of blood, a formula conveniently employed is as follows

$$\frac{O}{U} \times 100 = M$$

when  $O$  = cc of oxygen absorbed per minute

$U$  = volumes per cent of oxygen utilized

$M$  = minute output of the heart in cubic centimeter

In table 3 are shown the oxygen consumed and the carbon dioxide produced per minute, as measured by the Tissot method, the heart rate per minute, the oxygen utilization and the carbon dioxide accumulation in the blood, and the blood flow as calculated from these data For reasons pointed out in our previous paper, the circulation minute volume calculated from the oxygen figures is considered more reliable than that calculated from the carbon dioxide figures Therefore, in three experiments in which there was discrepancy between the two and in which there was reason to believe that the fault lay with the carbon dioxide results, only the oxygen figures are included

It is seen that most of the subjects, under the conditions of the experiment, gave figures for the output of the heart per minute and per beat which agree well with each other It may be said that the usual minute out-put of the normal resting adult is 3,500 to 4,500 cc., and the usual out-put per beat is 60 to 70 cc But there are exceptions and one very satisfactory experiment (Subject Bu) is a marked exception This individual had a minute volume of 6,780 cc. and an output per beat of 103 cc, in spite of the fact that his basal metabolic rate was low rather than high It is clearly necessary, therefore, to bring evidence as to the constancy or variability of the volume flow

in a given individual under similar conditions but at different times Table 4 shows the results of successive studies on two individuals,

TABLE 3  
*Blood flow in normal resting adults*

Subject	Oxygen consumed per minute	Oxygen utilization	Minute output	Heart rate per minute	Output per beat	Carbon dioxide produced per minute	Carbon dioxide accumulation	Minute output	Output per beat
	cc	vol per cent	cc		cc.	cc	vol per cent	cc	cc
A	231	6 53	3,520	54	65	175	4 69	3,730	69
R	236	5 97	3,950	68	58	200	4 86	4,120	61
H	230	5 59	4,120	64	64	187	4 50	4,160	65
F	243	5 70	4,270	64	67	176			
B	212	4 89	4,340	64	68	150	3 37	4,450	70
L	235	5 25	4,480	64	70	176	3 82	4,610	72
G ♀	192	4 28	4,490	65	69	156	3 26	4,790	74
M	249	5 45	4,570	66	69	189			
P	240	5 16	4,650	56	83	187	3 70	5,050	90
K	245	4 09	6,000	68	88	175			
Bu	213	3 14	6,780	66	103	159	2 34	6,800	103

TABLE 4  
*Successive determinations of two individuals, all under standard resting conditions*

Date	Minute output	Output per beat
Subject R		
	cc	cc.
March 31, 1923	3,700	59
April 17, 1923	3,940	60
April 19, 1923	3,950	58
June 2, 1923	3,960	55
March 13, 1924	3,760	57
Subject B		
January 4, 1924	6,780	103
January 7, 1924	6,260	100
March 7, 1924	4,540	73
March 10, 1924	5,340	83

one of whom had the largest blood flow in table 3 This individual showed wide variation, his minute volume being 6,780 on one occasion and 4,540 on another, with a change in the output per beat from 103

to 73 cc This variation was not associated with a change in pulse rate, metabolic rate, activity, or external temperature The other subject showed a remarkable constancy of minute volume and output per beat over a period of a year

Quantitative studies of such a matter as the output of the heart, which is controlled by factors not well understood, are better studied as individual results than as averages Many determinations of the venous oxygen tension of Subject Bu indicate that his usual resting blood flow is 6,000 to 7,000 cc although occasionally it may fall

TABLE 5  
*Blood flow compared with other measurements*

Subject	Minute output				Respiratory minute volume Circulatory minute volume	Output per beat		
	Total	Per square meter body surface	Per kilogram body weight	Per 100 cc. oxygen absorbed		Total	Per square meter body surface	Per kilogram body weight
	cc	cc	cc.	cc.		cc.	cc.	cc.
A.	3,520	1,970	53	1,525	0 90	65	36	1 1
R.	3,950	2,000	45	1,670	0 99	58	29	0 7
H.	4,120	2,190	62	1,780	0 98	64	36	1 0
F.	4,270	2,400	64	1,750	0 82	67	38	1 0
B.	4,340	2,320	64	2,050	0 66	68	36	1 0
L.	4,480	2,500	72	1,900	0 67	70	39	1 1
G ♀	4,490	2,750	90	2,340	0 60	69	42	1 4
M.	4,570	2,430	67	1,830	0 90	69	37	1 0
P.	4,650	2,470	63	1,940	0 68	83	44	1 1
K.	6,000	3,470	100	2,450	0 55	88	51	1 5
Bu	6,780	3,720	103	3,180	0 53	103	57	1 6

much below this The existence of such wide difference in different healthy people is corroborated by a scrutiny of earlier work The careful studies of Douglas and Haldane (1922), for example, demonstrated an output per beat of 128 cc in one subject and of 66 cc in another, under similar conditions, and these differences were quite constant in many determinations

In an effort to study the factors controlling the cardiac output, the minute volume and the output per beat of these normal subjects have been compared with some other measurements



in a given individual under similar conditions but at different times Table 4 shows the results of successive studies on two individuals,

TABLE 3  
*Blood flow in normal resting adults*

Subject	Oxygen consumed per minute	Oxygen utilization	Minute output	Heart rate per minute	Output per beat	Carbon dioxide produced per minute	Carbon dioxide accumulation	Minute output	Output per beat
	cc.	vol per cent	cc		cc	cc	vol per cent	cc	cc
A	231	6 53	3,520	54	65	175	4 69	3,730	69
R	236	5 97	3,950	68	58	200	4 86	4,120	61
H	230	5 59	4,120	64	64	187	4 50	4,160	65
F	243	5 70	4,270	64	67	176			
B	212	4 89	4,340	64	68	150	3 37	4,450	70
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March 13, 1924	3,760	57
Subject B		
January 4, 1924	6,780	103
January 7, 1924	6,260	100
March 7, 1924	4,540	73
March 10, 1924	5,340	83

one of whom had the largest blood flow in table 3 This individual showed wide variation, his minute volume being 6,780 on one occasion and 4,540 on another, with a change in the output per beat from 103

to 73 cc This variation was not associated with a change in pulse rate, metabolic rate, activity, or external temperature The other subject showed a remarkable constancy of minute volume and output per beat over a period of a year

Quantitative studies of such a matter as the output of the heart, which is controlled by factors not well understood, are better studied as individual results than as averages Many determinations of the venous oxygen tension of Subject Bu indicate that his usual resting blood flow is 6,000 to 7,000 cc although occasionally it may fall

TABLE 5  
*Blood flow compared with other measurements*

Subject	Minute output				Respiratory minute volume Circulatory minute volume	Output per beat		
	Total	Per square meter body sur- face	Per kilogram body weight	Per 100 cc. oxygen absorbed		Total	Per square meter body surface	Per kilogram body weight
	cc.	cc	cc.	cc.		cc.	cc.	cc
A.	3,520	1,970	53	1,525	0 90	65	36	1 1
R	3,950	2,000	45	1,670	0 99	58	29	0 7
H	4,120	2,190	62	1,780	0 98	64	36	1 0
F	4,270	2,400	64	1,750	0 82	67	38	1 0
B	4,340	2,320	64	2,050	0 66	68	36	1 0
L	4,480	2,500	72	1,900	0 67	70	39	1 1
G ♀	4,490	2,750	90	2,340	0 60	69	42	1 4
M	4,570	2,430	67	1,830	0 90	69	37	1 0
P	4,650	2,470	63	1,940	0 68	83	44	1 1
K.	6,000	3,470	100	2,450	0 55	88	51	1 5
Bu	6,780	3,720	103	3,180	0 53	103	57	1 6

much below this The existence of such wide difference in different healthy people is corroborated by a scrutiny of earlier work. The careful studies of Douglas and Haldane (1922), for example, demonstrated an output per beat of 128 cc in one subject and of 66 cc in another, under similar conditions, and these differences were quite constant in many determinations

In an effort to study the factors controlling the cardiac output, the minute volume and the output per beat of these normal subjects have been compared with some other measurements

In table 5 it is seen that neither the minute output nor output per beat has any exact or noteworthy relation to body weight, to body surface area, or to the volume of oxygen absorbed. The net respiratory minute volume<sup>1</sup> has not varied directly or inversely with the circulatory minute volume. There is of course no doubt that the volume of blood flow is a function of metabolic rate, but it is equally clear that there are other factors of potent if subsidiary influence. A study of a series of normal adults such as this, shows that even with favorable and, in a sense, trained subjects—so that basal conditions were well maintained—and with earnest efforts to secure identical surroundings in successive experiments, the minute volume may vary widely in different subjects. Even on the same subject at different times the output of the heart per minute may vary as much as 30 per cent.

#### COMMENT

Our figures for the minute output of the heart and the output per beat are smaller than many appearing in the literature. This may be due in part to our insistence upon rest and fasting. Even so our figures lend support to the view that the output of the heart per beat is not fixed but variable, since an output of 60 to 70 cc per beat is not enough to transport the large volume of oxygen required during strenuous exertion, even at very rapid heart rates. Observations bearing on this problem are reserved for a subsequent paper.

The significance of the variations in volume flow in Subject Bu is not clear. As the broad physiological viewpoint of Haldane (1922) has emphasized, one object of changes in the volume of blood flow is to maintain the optimum condition of gas pressure in all parts of the body. Changes in blood flow without change in total metabolism suggests the influence of some other factor, or the redistribution of the blood in various parts of the body.

#### SUMMARY

In a series of normal adults at complete rest the volume of blood expelled by the heart has varied from 3,500 cc per minute and 58 cc per beat to 6,800 cc per minute and 103 cc per beat.

<sup>1</sup> The net respiratory minute volume has been calculated from the total amount of air expired per minute with an allowance of 130 cc per respiration for the dead space.

In one individual, tested repeatedly over a period of one year, the circulatory minute volume varied only from 3,700 to 3,960 cc. In a second individual, during a period of two months the circulatory minute volume varied from 6,780 to 4,540 cc.

The significance of these findings is discussed. The oxygen and carbondioxide content of the arterial and of the "mixed venous" blood have been determined in the series of eleven adults, and the results are given.

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# EVIDENCE THAT DIGITALIS INFLUENCES CONTRACTION OF THE HEART IN MAN

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## HISTORICAL<sup>1</sup>

Since 1785, when digitalis was first used systematically in the treatment of human disease, the criteria which have been employed in the attempt to define precisely when the drug is indicated, have undergone changes depending on those aspects of the study of the heart which were current at the time. These 140 years fall almost naturally into distinct periods. At first a belief initiated by Withering (1785) in the efficacy of digitalis as a diuretic was general and almost unquestioned. His chief indication for the exhibition of the drug is clear from this description: "Further experience convinced me, that the *diuretic* effects of this medicine do not at all depend upon its exciting a nausea or vomiting, but, on the contrary, that though the increased secretion of urine will frequently succeed to, or exist along with these circumstances, yet they are so far from being friendly or necessary, that I have often known the discharge of urine checked, when the doses have been imprudently urged so as to occasion sickness."

But Withering himself could make no distinction between edema due to failure of the heart and edema due to failure of the kidneys though he was well aware that it exercised "a power over the motion of the heart," that was naturally impossible before the days of Hope and of Bright. The ground for the belief in the efficacy of the drug as a diuretic rested, however, securely on prolonged and detailed observation. Opinion in later years departed from this early belief on grounds which, as can be seen now, were scarcely sufficient substitutes for direct experience. Withering's observations were confirmed, and subsequently expanded by such observers as Ferriar (1799), Kinglake (1801), and Beddoes (1801) who noted that digitalis slows the pulse and strengthens it.

In addition to its action in causing diuresis, another important effect of the drug was noticed. This was described by Kreysig (1814) who concluded that digitalis must contribute something to the energy of the heart, on account of the improvement which could be seen following its use. Throughout the century this "some-

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<sup>1</sup> Use has been made in the survey of excellent papers by A. W. Meyer (1912), A. R. Cushny (1911), and G. C. Robinson (1922).



thing" was frequently called "energy" by which was probably meant what would now be called an increase in contractile power. Not infrequently reference was made to the effect which digitalis exerted on edema. This effect was considered as the result of its action sometimes on the vessels, sometimes on the heart as part of a general ill defined influence (Einhorn).

Meanwhile, for reasons which we have not traced, digitalis fell into discredit and disappeared from use. Corvisart (1806-1818) for instance, does not even mention the drug in any of the editions of his book. Perhaps the indiscriminate indications given for its employment as for example in tuberculosis, scarlet fever, measles and hemorrhage, alienated the respect of physicians. Hope (1839), Boullaud (1835), and Stokes (1853), even used it as a sedative. At all events the neglect continued and reasons justifying it were supplied later by Traube (1871) and by Corrigan (1832) on the basis of the discoveries of Laennec (1821) and Hope.

These discoveries provided the background for those discussions with which clinicians were occupied for the major part of the 19th century. Laennec's contribution consisted, as is universally known, in making it possible, during life, to detect sounds arising during the heart's action, Hope in 1831 demonstrated by experiments that these sounds are caused by the motion of the valves, and so made it possible to ascertain whether their behavior was normal. He also showed that incompetence of the aortic valves resulted in regurgitant murmurs. Perhaps as a consequence of these observations, Corrigan (1832) was able to describe the disease known as aortic insufficiency. In his original paper, he discussed the action of digitalis in this affection. This he thought was twofold, first that it was a cardiac sedative, an idea which he derived from Bertin and Boullaud (1824) and perhaps even from Withering (1785), and second, that it slowed the pulse. He expressed his views as follows: "Having laid down the plan of treatment proper to be adopted as far as it produces effects upon the system, and through it upon the heart constituting a part of the system, it now remains to examine the propriety of employing in this disease a remedy such as *digitalis*, which produces a specific effect upon the heart rendering its action slow and weak, and which in consequence of that effect is usually recommended in cases of heart disease in conjunction with the measures already deprecated. In inadequacy of the aortic valves the pulse generally ranges from 90 to 110. After each contraction of the ventricle during the pause or interval of rest occurring between that contraction and the next following, a quantity of blood is regurgitating into the ventricle. The danger of the disease is in proportion to the quantity of blood that regurgitates, and the quantity that regurgitates will be large in proportion to the degree of inadequacy of the valves, and to the length of pause between the contractions of the ventricle during which the blood can be pouring back. If the action of the heart be rendered very slow, the pause after each contraction will be long, and consequently the regurgitation of blood must be considerable. Frequent action of the heart, on the contrary, makes the pause after each contraction short, and in proportion as

the pauses are shortened, the regurgitation must be lessened. Instead, then, of regarding an increase of frequency in the action of the heart as an aggravation of the disease, it must be viewed, as we have already viewed hypertrophy of the heart, as a provision for remedying as far as possible the evil consequences arising from inadequate valves. To retard in such circumstances the action of the heart would be to do an injury. In every case of this disease in which *digitalis* has been administered, it has invariably aggravated the patient's sufferings."

This statement by Corrigan set the fashion for subsequent speculation. From then until now there has been continuous discussion as to the indication for giving *digitalis* when the heart valves are diseased. It is interesting to notice that in this first paper on aortic insufficiency, the harm which *digitalis* can do in the condition, was recognized and vigorous objection to its use was taken.

The general discredit into which *digitalis* fell continued until about 1840. Then it began again to be used in the treatment of heart disease in Germany by Schoenlein (1842) and Traube, and in France by Aran (1842). In England, distrust of the drug continued. Stokes (1853), Latham (1847), Walshe (1854) and Fothergill (1871) followed Corrigan's view. Diagnosis based on deductions drawn from auscultation accordingly dominated the discussion. The factors which were considered to be determining were its effect on the rate of the pulse and its effect on the "energy" of the heart. There was general belief in its relative advantage in stenoses, its disadvantages in insufficiencies, its beneficial employment in mitral regurgitation, its harmfulness in aortic insufficiency. So far as the effect on the pulse rate is concerned, it was clear that no harm could be done when the mitral valves were involved, injury resulted only when insufficiency of the aortic valves existed. This discussion is, quite properly, not yet ended. Valvular disease was in 1912 still made the basis, at least in part, for deciding on the administration of *digitalis* in Krehl's clinic (A. W. Meyer, 1912).

It must have been Traube (1861) who furthered the view, as a result of his studies on the stimulating action of *digitalis* on the vagus nerves, that *digitalis*, because it slowed the heart, was sedative and accomplished its effects by quieting the heart.<sup>2</sup> Whether giving *digitalis* increased or diminished the "energy" of the heart, became then a second matter for discussion. Fothergill (1871), Nothnagel (1878), Leyden (1881), Fränkel (1882) and Balfour (1898) in part, advocated its use, on the ground that it had this action irrespective of the presence of any valvular lesion whatsoever.

It is profitless to attempt to trace, through the writings of the distinguished clinicians of the century, the precise position which each in turn held in respect to these problems. The criteria that were available were based on auscultation, on pulse rate, and on diuresis. The effect of *digitalis* on rhythm, blood pressure and the size of the heart could naturally not be estimated. The chief factor in making a decision was preëminently the state of the valvular lesion,

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<sup>2</sup> This inference was in all probability drawn from the study of cases which were the subject of auricular fibrillation.

and to a less extent, because it could be so inadequately estimated, the condition of the muscle, other factors were the pulse rate, diuresis, and, when present, such symptoms as palpitation and irregularity,<sup>3</sup> and finally, eventualities such as are contingent on the state of the cerebral vessels and their ability to bear increased pressure. That the blood pressure was raised by digitalis physicians believed, because this effect was observed in experiments on animals.

An important new development occurred when Boehm (1872) emphasized the specific effect of digitalis on heart muscle in frogs. Traube, in all probability, as Cushny points out, missed this action because he utilized only mammals in his experiments. In frogs, however, both Boehm and Schmiedeberg easily recognized this action. The actual effects on contraction were discovered in later experiments especially those of Roy and Adams (1892), and of Cushny (1897).

The influence of this teaching became apparent almost at once in works on clinical medicine.<sup>4</sup> It is apparent in the writings of B. Bramwell (1884) and of G. A. Gibson (1898), who applied the results of experiments directly to phenomena observed in man, without attempting to notice whether the experimental data paralleled the events in human physiology (p. 280). A more critical, almost contemporary attitude, was taken by Balfour (1898) (p. 104 ff.). At the turn of the 19th century, the way was thus paved by Krehl (1901) and Romberg (1906), for a study of the significance of the behavior of muscle in heart disease, and likewise of the effect of digitalis upon the heart muscle under clinical conditions.

That the data obtained by pharmacologists were not applicable directly to human conditions was clear from the fact that the blood pressure does not rise in human beings when they are under the influence of digitalis. Both Gottlieb and Sahli admitted this fact at the German Medical Society in 1901. But although, at that time, the blood pressure in patients could be satisfactorily measured, no method was available for studying the effect of digitalis on the heart muscle itself. It was assumed then, and also later, on the basis of such experiments as those of Ionescu and Loewi (1908), of Kasztan (1910), Fahrenkamp (1911), Gottlieb and Magnus (1902), and Joseph (1913) that, although no influence on the general blood pressure resulted from peripheral effects, such as the dilator action of the drug on the kidneys, and its constrictor action on the intestine, the efficacy of the drug was to be explained, nevertheless, through some such remote actions rather than from an influence on heart muscle. On peripheral vessels in man, Vagt (1909) and Eychmuller (1909) found no effect. And so the matter stands.

Meanwhile, stimulated by the researches of Gaskell and Engelman on the differentiation of the properties of heart muscle, a great step forward was initiated in 1902 by the work of Mackenzie and Wenckebach. The striking effect which digitalis was found to have in reducing the rate of the completely irregular pulse of auricular fibrillation, together with studies of the effect of the drug on other

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<sup>3</sup> The precise mechanism was of course not diagnosticated.

- W. Balfour, Clinical Lectures on Diseases of the Heart and Aorta, 1st

forms of irregular heart action, deflected attention away from investigations which seemed in 1901 about to be carried forward. So great was the interest in the effect of digitalis on the cardiac irregularities, that the view arose that it was preeminently the irregular heart on which digitalis acted (Mackenzie, 1911). That it was especially indicated under these circumstances seems to have been clear to Jürgensen (1899). This belief was enforced by the observation that when the mechanism of the heart was normal no influence on the rate was to be observed (Cohn 1915 a), except in heart failure when edema was present, or in what has come to be known as the hypodynamic heart (Cohn, 1915 a).

Attention was again directed to the action of digitalis on human heart muscle when evidence was obtained in electrocardiograms that changes in the curves followed on taking the drug (Cohn, 1915b). These changes, which consisted in alterations in the form of the T-wave, could so far as knowledge of the genesis of action currents is concerned, have arisen only through action on the muscle. This was the evidence in 1913 from which an effect on the human heart muscle could most directly be inferred. So strong is the belief in the inefficacy of digitalis, except in irregular heart action, that it has seemed wise actually to call attention to the fact that giving the drug aids in eliminating edema when the rhythm is regular.

The state of knowledge then, so far as human physiology is concerned, may be described in this fashion. Digitalis is known to act in man on the centers of the *vagus nerves*. It is known likewise that digitalis does not raise the general *blood pressure*. It is assumed on the basis of Cushny's (1897) observation on mammals that an *effect on contraction* of the heart muscle takes place in man, as it was observed in his experiments on mammals which were performed with moderate though not exactly known doses of the drug. This assumption is also held on the basis of such experiments on mammals as those of de Heer (1912), with doses far greater than those used in patients, and as the result of the analysis by Lewis, Drury and Iliescu (1922), of the effect of strophanthin on the mechanism of the contractile act, in the course of which they found that a "widening in the refractory period" took place. It is assumed that *edema-fluid* is eliminated on the basis of such experiments as those of Joseph (1913), in which the dose given was comparable presumably with that employed in the clinic.

But so far no studies have been made directly on the effect of digitalis on the contraction of the heart muscle in man. Such investigations are here reported.

## METHOD

In attempting this investigation two methods were open to us. The first consisted in making x-ray photographs in rapid succession of the whole heart, and recording the changes in size as the heart moves from the diastolic to the systolic position (Groedel (1909) and Dessauer (1912)). This method was developed first. Eyster and Meek (1920) extended its use. They obtained radiographs of the human heart at known instants in the cardiac cycle, by recording on electrocardiograms the time at which the radiographs were taken. From data so obtained, they calculated by the use of Bardeen's (1918) formula the volume of the heart in systole and diastole, and from the differences between these two measurements obtained the volume output of the heart per beat.

The second method introduced by Gott and Rosenthal (1912)<sup>5</sup> consisted in photographing, with Roentgen rays, the excursion of points of the two borders of the heart. By this procedure continuous curves were obtained. Becker (1914) and Crane (1916) each made use of an apparatus similar in principle and recommended its use particularly in the study of irregularities.

The apparatus we employed resembles that of Gott and Rosenthal (figs 1 and 2). We selected this method, first because of its greater simplicity and the greater ease with which the requisite apparatus could be constructed, secondly, because of the ease of obtaining the records, thirdly, because the records are in the form of curves which can easily be obtained in long strips throughout a whole respiratory cycle, and fourthly, because the curves obtained represent the shortening or contraction undergone during systole by that portion of the left ventricular margin that is photographed, this being exactly the effect of digitalis that concerns us. The fact that it was not possible to calculate the volume output of the heart from the measurements we obtained of the curves, did not seem to us to be a disadvantage, for the figure obtained for the volume output calculated from Bardeen's formula from data based on radiographs are said to

<sup>5</sup> These authors call attention to the description in Polish by Sabat of an apparatus like theirs. Their own was however developed independently and without knowledge of that of Sabat.

be accurate only within 10 per cent Since this is about the increase in output we anticipated as the result of administering digitalis, the advantage of the method is naturally lost

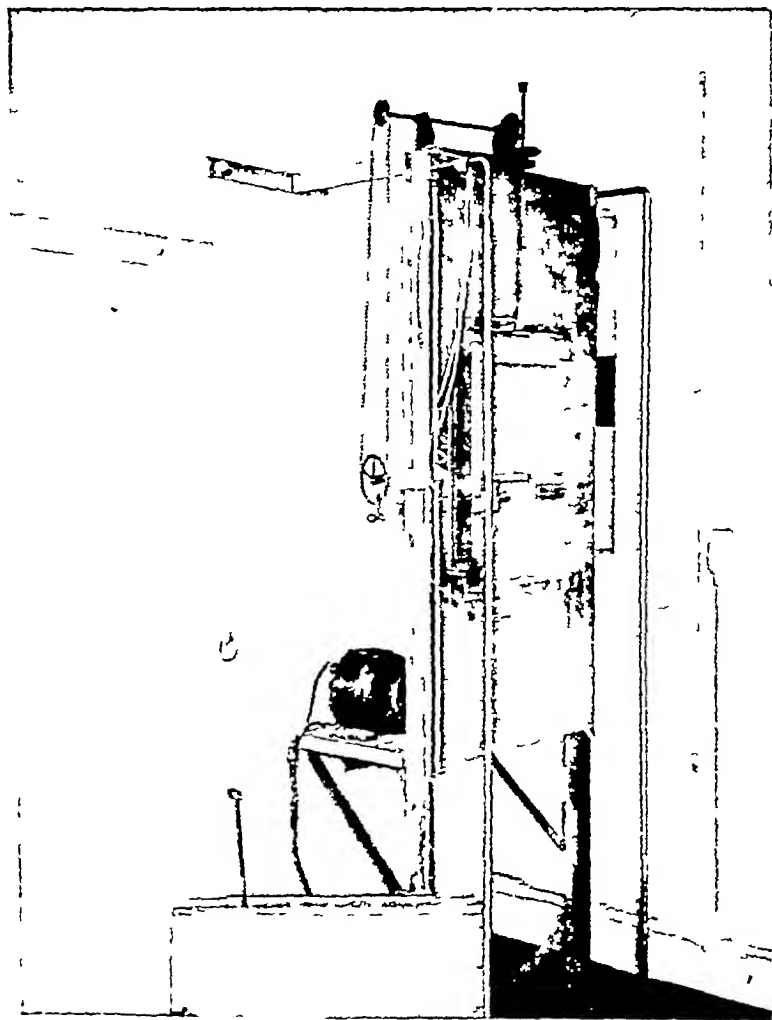


FIG 1 PHOTOGRAPH OF THE APPARATUS USED IN MAKING MOVING X-RAY  
PHOTOGRAPHS OF THE HEART

See text for description of parts and figure 2 for a diagrammatic sketch of the apparatus

In practice<sup>6</sup> the patient faces a lead screen in which a transverse slit 0.5 or 1 cm wide is cut. The level of the slit is adjustable. It is usually placed opposite that portion of the border of the left ventricle which exhibits the greatest excursion. The x-ray tube is placed behind the patient 36 inches from the lead screen. On the other side of the lead screen a cassette, which carries an x-ray film, is raised by a motor past the slit. Between the patient and the lead

Diagram of X-Ray Apparatus

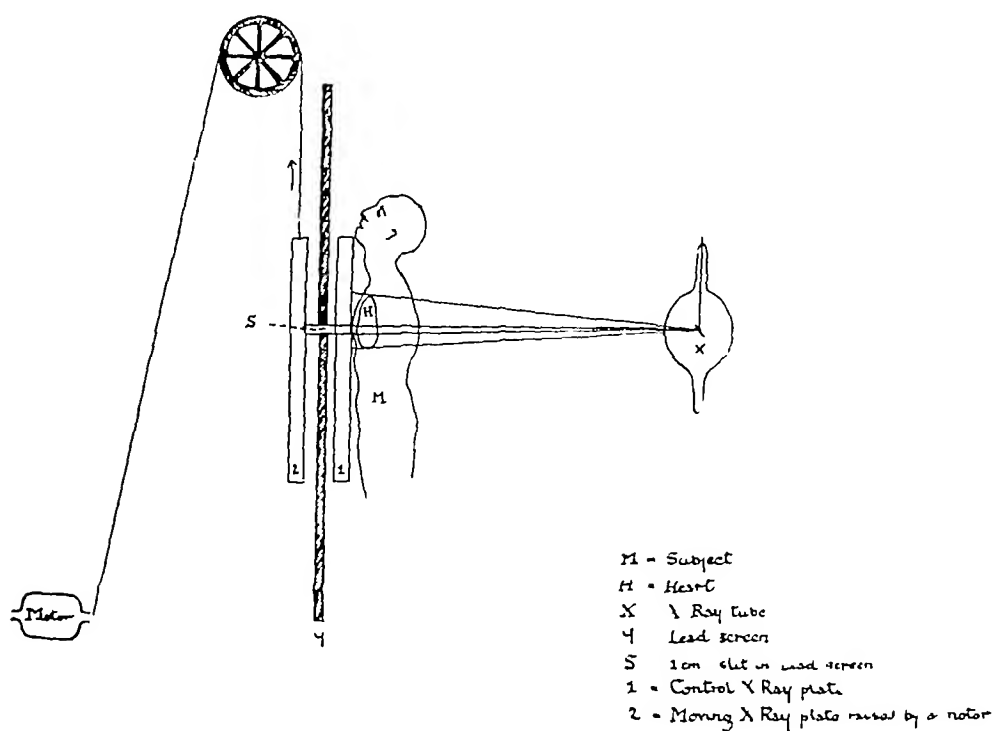


FIG. 2 SCHEMATIC DRAWING OF THE X-RAY APPARATUS ILLUSTRATING THE METHOD OF EXPOSING THE X-RAY FILMS

screen another cassette is placed, which also holds an x-ray film. This is the control film and is stationary. On this it is possible, by a device next to be explained, to record the portion of the border of the heart of which the curve is made. This portion is identified by the shadows cast by two levers, which swing at the level of the slit between the patient and the stationary film. One lever records the

<sup>6</sup> The authors are deeply indebted to Miss Christine Macdonald of the X-ray Department of the Institute for her technical cooperation in this investigation.

time in seconds, the other the respiration. The time recorder is the armature of a magnet in series with a Petzold clock. The respiratory movements are communicated to the lever of a piston-recorder by a Politzer bag bound against the chest by a muslin binder. (See figs 1 and 2.)

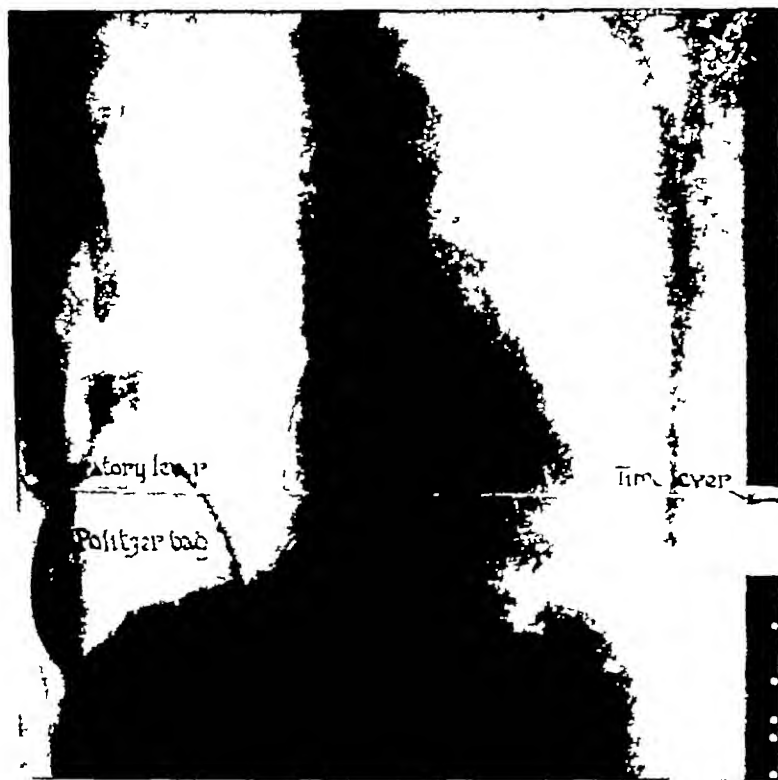


FIG 3 CONTROL PLATE SHOWING LEVEL OF THE RIGHT AURICULAR, AND OF THE LEFT VENTRICULAR MARGINS OF WHICH THE MOVING X-RAY FILMS WERE OBTAINED

The control film was made first (fig 3). Then without changing the patient's position the moving film was exposed. The records are similar to a sine-curve, the trough representing systole and the crest diastole (fig 4). The distance between the two positions was used as a measure of the degree of ventricular contraction or shorten-

<sup>7</sup> Eastman duplitzed films were used in a Reenforced French Cassette



ing No attempt, as has been said, was made to infer volume changes from this simple linear measurement The films were exposed during normal quiet breathing Only those films were measured in which it was clear, from the controls, that identical points of the heart's margin had been photographed That the points were identical was shown by actual superposition of the control films, as well as by comparison of the measurements made of them The measurements were not corrected to obtain values comparable with those taken at a distance of 2 meters, the conclusions which we draw are independent of the magnification caused by the divergence of the x-rays We appreciate the fact that an error might result if the size, that is to say the transverse diameter, of the heart decreased after giving digitalis In this case, the greater the decrease, the less will be the magnification of the excursion If we correct our results in accordance with this error, we increase the magnitude of the change we found Measurements were taken of the control film, of the original curve (moving film), and of a tracing made of the moving film All the waves in a respiratory cycle were measured and the average of these was used

#### OBSERVATIONS

In order to obtain satisfactory curves, we found it advantageous to limit the choice of patients to men, and especially men whose chests were reasonably thin We found it desirable, also, to select men who were free from edema This precaution we took because of the uncertainty which still prevails on the subject of the blood volume, even if this can be measured accurately, when fluid is shifted in the body from the tissues to the blood and from the blood to the urine It was somewhat difficult to obtain suitable patients For this reason a large series of cases is not available on which to base the subsequent study The care with which the patients were selected, and the accuracy with which the observations were made, justify us, we think, in presenting the results of our study We studied the curves of 5 patients To 4

#### FIG 4 MOVING X-RAY FILM

The manner in which records like this were obtained is described in the text (a) was taken 2-14-24, before, (b) was taken 2-18-24, after the administration of digitalis 1.2 gm, and (c) was taken 2-28-24, after the effect had worn off



FIG 4a

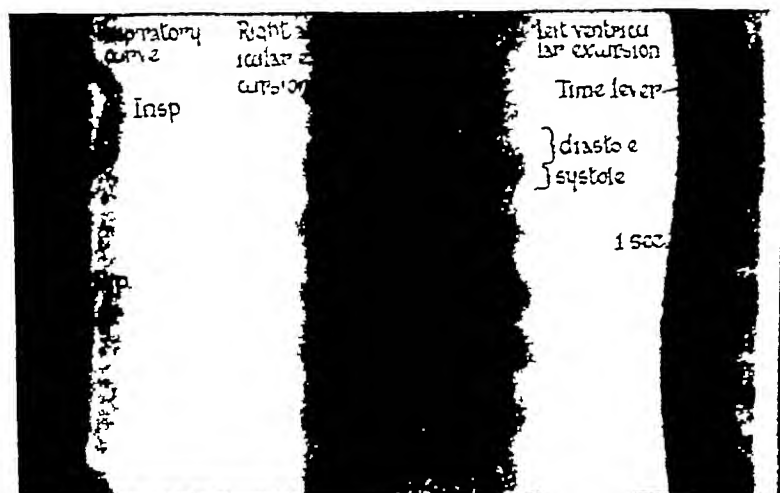


FIG 4b

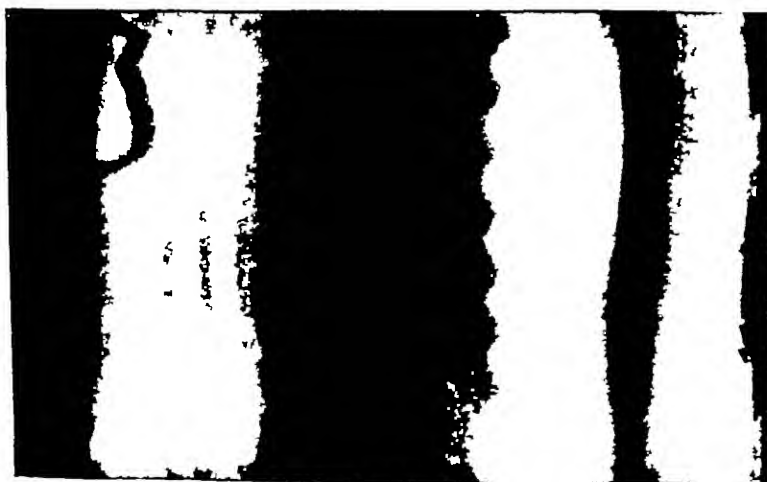


FIG 4c

digitalis was given, one served as a control. The hearts of two patients exhibited the normal rhythm, the hearts of the other two were the subjects of auricular fibrillation. Curves were, of course, taken both before digitalis was given and again after an amount had been given sufficient to produce a therapeutic effect clinically, and to bring about changes in the T-wave in the electrocardiogram. In three patients the observations were repeated.

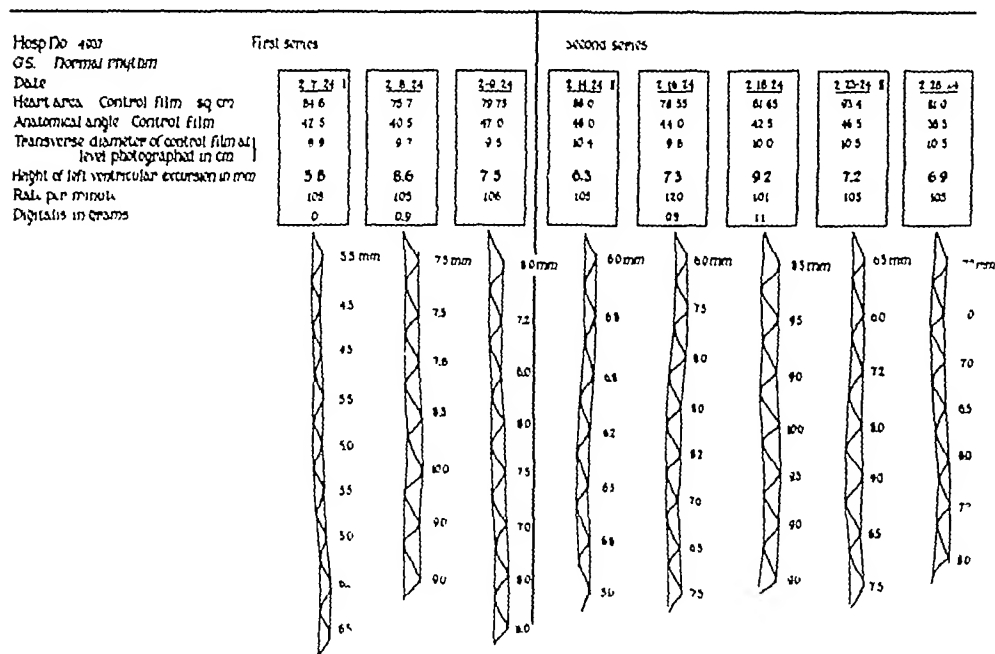


FIG 5 In this figure and in figures 6, 8 and 9, the curves illustrated are tracings of the left ventricular excursion made from the original films as described in the text. At the head of each curve, in the oblong box are given measurements corresponding to the curves below.

*Case 1* G.S., Hosp No 4937, was a male, 24 years old. He complained of "heavy beating" of his heart for 5 years and of "asthmatic" attacks for 4 years. He had influenza 5 years ago, followed by dyspnea on exertion and palpitation. The action of the heart was said to be irregular so that he was given digitalis. Four years ago he had an attack of bronchitis which later changed character and became "asthmatic."

On physical examination his heart was small and of the pendular form. There were no murmurs. There were numerous auricular premature contractions. The lungs appeared to be normal. The blood pressure was 100-122 systolic and 70-84 diastolic. There was no edema. The Wassermann reaction was negative.

TABLE 1  
G. S. Hospital No. 1937 Normal rhythm

Date	Intercostal space photographed	Transverse diameter of control film at level photographed	Analysis of moving film								Blood pressure		Analysis of control film						mg Digitalis	
			Transverse diameter of original in inspiration				Transverse diameter of tracing in inspiration		Left ventricular excursion	Right auricular excursion	Rate per minute	Systolic	Diastolic	Area heart	Middle line to left border	Middle line to right border	Transverse diameter	Long diameter		Anatomical angle
			Systole		Diastole		Systole	Diastole												
			cm	mm	cm	mm			mm	mm	mm Hg	mm Hg	sq cm	cm	cm	cm	cm	degrees		
2-7-21	51 S	9.9	10.2	11.2	10.1	11.0	5.8	1.6	108	100	80	84.6	7.7	2.8	10.5	12.6	12.5	0.9		
2-8-21	51 S	9.7	10.2	11.2	10.0	11.4	8.6	1.6	105			75.7	7.4	2.6	10.0	11.6	10.5			
2-9-21	51 S	9.5	10.2	11.1	9.8	11.1	7.5	5.0	106	120	75	79.8	6.7	3.3	10.0	12.2	47.0			
2-12-21																				
2-11-21(II)	51 S	10.1	10.5	11.7	10.6	11.6	6.3	1.5	105			88.0	7.7	2.8	10.5	12.6	18.0	0.9		
2-16-21(II)	51 S	9.8	10.2	11.3	10.3	11.5	7.3	1.4	120	121	85	78.6	7.2	2.8	10.0	12.1	14.0			
2-17-21																				
2-18-11	51 S	10.0	10.2	11.3	10.0	11.7	9.2	7.1	101			81.7	7.1	3.3	10.1	12.3	12.5	1.1		
2-20-21										121	70									
2-23-21(II)	51 S	10.5	10.6	11.8	10.7	12.2	7.2	6.2	105	121	76	93.1	7.5	3.5	11.0	13.8	16.5			
2-28-21	51 S	10.5	10.5	11.5	10.7	11.8	6.9	5.2	105		74	81.0	7.7	3.1	10.8	12.1	38.5			
2-29-21										120										

The urine was normal. At first, unusual slowing followed the administration of digitalis (0.9 gm), and the number of auricular premature contractions which had been present decreased. He experienced no attacks of asthma while in the hospital and none since leaving, he was moved away from his home where there were cats and dogs. It was these which caused the attacks of asthma.

On February 7, 1924, the left ventricular excursion in this patient was 5.8 mm in the 5th interspace (table 1, figs 4 and 5). On February 8, after giving digitalis 0.9 gm, the excursion increased to 8.6 mm and on February 9, fell to 7.5 mm, the rate remaining unchanged during these three observations. On February 14, in a second series of observations before giving digitalis the excursion measured 6.3 mm. On February 16, after taking digitalis 0.9 gm, the excursion increased to 7.3 mm, but later, on February 18, after a total of 1.1 gm had been given, the excursion increased still farther to 9.2 mm. At this time a marked change in the T-waves of the electrocardiogram was observed. The administration of digitalis was discontinued on February 18, on February 23, the excursion decreased to 7.2 mm and on February 28, to 6.9 mm. No significant changes occurred either in heart rate or in blood pressure which need be considered in accounting for the difference in the heights of the excursions observed.

*Case 2* A. F., Hosp. No. 4814, was a male, 34 years old. He complained of nervousness, fatigue and palpitation for 6 months. He had suffered from scarlet fever in childhood and influenza in 1918. He passed the army tests, but was told that he had a "little" albumen in the urine.

On physical examination the heart was found to be enlarged, a late systolic murmur was heard at the apex. The rhythm was regular. The blood pressure was 160-180 systolic and 90-100 diastolic. There was no edema. The phenol-sulphonaphthalein test was 45 and the Van Slyke index 27. The urine contained a small amount of albumen, red blood cells and casts. He could excrete water but was unable to concentrate the urine. The diagnosis was thought to be chronic glomerulonephritis.

Curves taken on October 5 and 11, at the level of the 5th interspace show excursions of 4.2 mm and 3.7 mm respectively (table 2, fig 6), the difference (0.5 mm) between them is within the experimental error. On October 20, after digitalis 2.3 gm was given, the excursion increased to 6.6 mm, and the T-waves showed slight changes. Digitalis was then discontinued. Eighteen days later, November 7, the

excursion of the same portion of the left ventricular margin decreased to 2.9 mm. In order to account for the change in the height of the excursion, aside from the effect produced by digitalis, two other factors must be considered as being possible contributors in bringing it about, first, the rate and second, the blood pressure. That the

Hosp No 4911

A.E. Normal rhythm

Date

Heart area - Control film sq cm

Anatomical angle Control film

Transverse diameter of control film (L)  
level photographed - in cm.

Height of left ventricular excursion in mm.

Rate per minute

Digitalis in grams

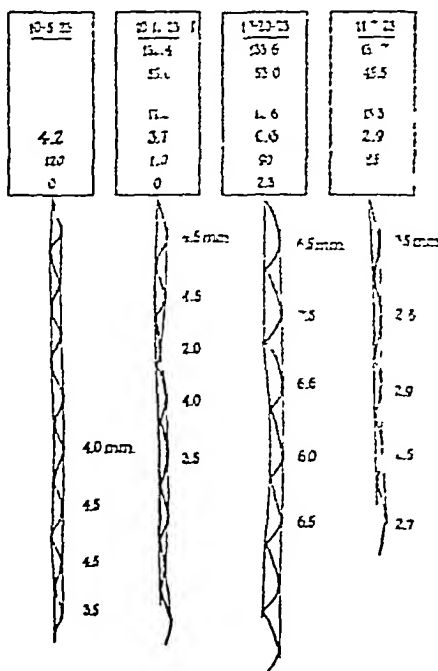


FIG 6 SEE FIGURE 5

decrease in rate was not a material factor is shown by the fact that on November 17, when the effect of digitalis had worn off the ventricular excursion decreased to 2.9 mm although the rate was approximately the same (85 per minute) as when the excursion was 6.6 mm. Nor, as the records show could changes in the blood pressure, either systolic or diastolic, be associated with the difference found (table 2, fig 6).

TABLE 2  
A F Hospital No 4814 Normal rhythm

Date	Intercostal space photographed	Transverse diameter of control film at level photographed	Analysis of moving film						Blood pressure		Analysis of control film						Digitalis gm	2 3	
			Transverse diameter of original in inspiration		Transverse diameter of tracing in inspiration		Left ventricular excursion	Right auricular excursion	Rate per minute	Systolic mm Hg	Diastolic mm Hg	Area heart sq cm	Middle line to left border cm	Middle line to right border cm	Transverse diameter cm	Long diameter cm			Anatomical angle degrees
			Systole cm	Diastole cm	Systole cm	Diastole cm													
10-4-23			12 7	13 3	12 6	13 5	4 2	4 2	120	160	100	132 4	9 3	3 2	12 5	14 5	55		
10-5-23																			
10-8-23																			
10-11-23 (I)	5 I S	12 5	12 7	13 7	12 7	14 0	3 7	Very deep	110	180	100								
10-20-23	5 I S	12 6	12 5	13 2	12 1	13 3	6 6	5 0	90	164	90	133 6	9 0	3 4	12 4	15 1	53		
11-7-23	5 I S	13 3	13 5	14 2	13 4	14 3	2 9	5 4	85			137 7	9 7	3 5	13 2	15 3	45		
11-8-23										176	90								

*Case 3* T. S., Hosp. No. 4390, was a male, 24 years old. He complained of cough and shortness of breath. He had rheumatism at 9 years. Four years later there was an attack of shortness of breath while working as a laborer. He was rejected by the Navy in 1917 because of heart trouble, the first intimation the patient had of the presence of this disease. In 1919 the first symptom, shortness of breath, occurred. In 1921 he was first admitted to hospital because of cough, edema and shortness of breath. Since then there have been three breaks in compensation.

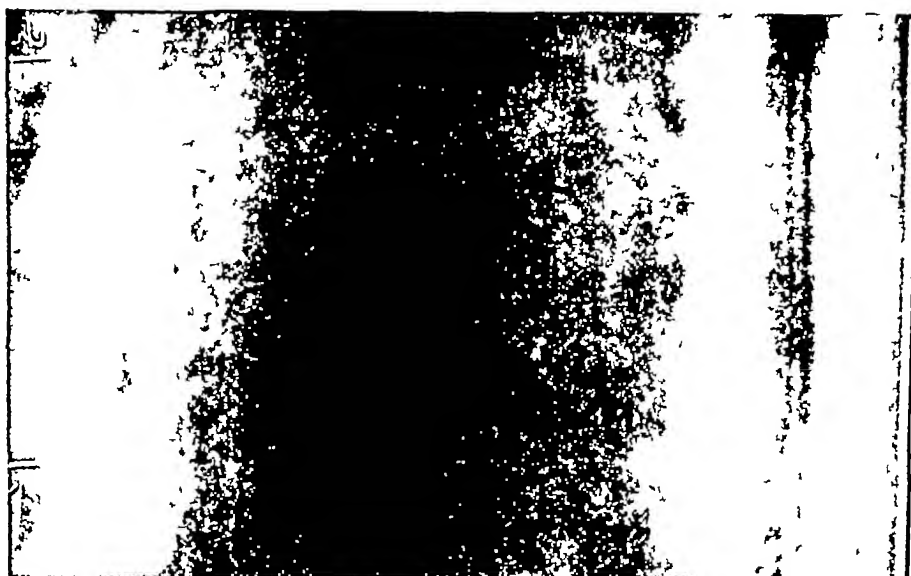
On physical examination auricular fibrillation was found to be present. The heart was enlarged. There were the signs of mitral stenosis and insufficiency, and in the electrocardiogram, of right ventricular preponderance. The blood pressure was 90-105 systolic and 50-70 diastolic. The urine was normal.

On November 16, after having received digitalis, the ventricular excursion was 8.5 mm in the 6th interspace, the rate being 100 per minute. The effect of this amount of digitalis was allowed to wear off. Then, on December 3, the ventricular excursion decreased to 4.6 mm, the rate being 115 per minute (table 3, figs. 7 and 8). Between December 3, and December 17, digitalis 5 gms. was given, the ventricular excursion again increased to 8.5 mm, the rate being 90 per minute. Although he continued to receive digitalis the ventricular excursion fell slightly to 8.3 mm on December 31. Coincidentally there was a slight decrease in rate (80).

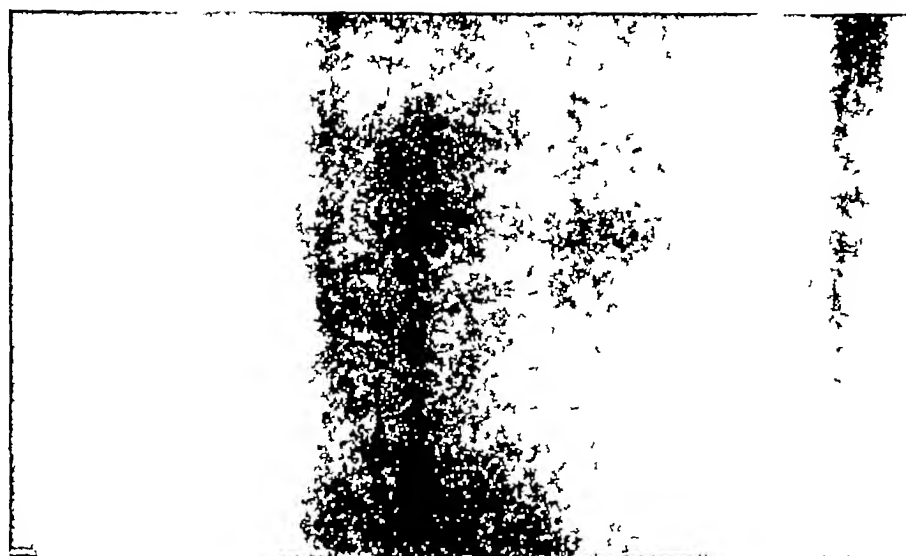
On the same days that the photographs were made in the 6th interspace, a parallel set was taken in the 5th interspace. The excursion at this point was 10.4 mm on November 16, then after the digitalis effect had worn off it decreased to 8.2 mm (December 3), but increased again when the patient was for a second time placed under the influence of digitalis to 12.7 mm (December 17) and 12.0 mm (December 31).

A comparison of the series in the 6th and 5th interspaces shows that difference in the heights of the excursion is found at different portions of the margin, and that in both series the effect of digitalis in increasing the ventricular excursion is equally exhibited. Attention is called in this case of auricular fibrillation to the total irregularity of the rhythm, and to the variation in the height of the excursions.





7a



7b

FIG 7 MOVING X-RAY FILM

(a) was taken 12-3-23, before and (b) was taken 12-17-23 after the administration of a total of digitalis 5.0 gm

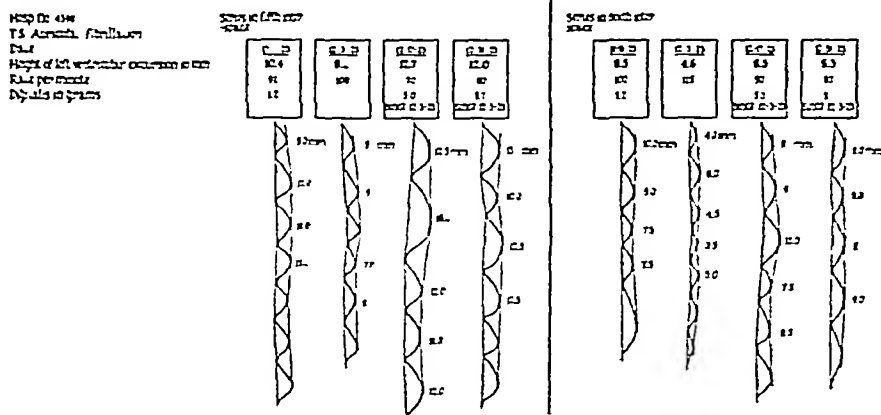


FIG 8 SEE FIGURE 5

TABLE 3  
T S Hospital No 4390 Auricular fibrillation

Date	Intercostal space photographed	Analysis of moving film		Blood pressure		Digitals
		Left ventricular excursion	Rate per minute	Systolic	Diastolic	
		mm		mm Hg	mm Hg	mm
11-15-23				100	60	
11-16-23	6 I S	8 5	100			
12- 3-23	6 I S	4 6	115	93	60	1 2
12-17-23	6 I S	8 5	90	86	58	5 0*
12-31-23	6 I S	8 3	80	90	60	8 7*
11-15-23				100	60	
11-16-23	5 I S	10 4	92			
12- 3-23	5 I S	8 2	100	93	60	1 2
12-17-23	5 I S	12 7	70	86	58	5 0
12-31-23	5 I S	12 0	80	90	60	8 7

\* Since December 3, 1923

Case 4 M H, Hosp No 4932, was a male, 20 years old. He complained of shortness of breath for 3 months. His illness began suddenly 3½ months before admission with precordial pain and blood streaked sputum following the severe exertion of lifting packing cases. He continued at work though he had slight cough and saw blood streaked sputum. Gradually dyspnea increased until 3 months ago (October 30) when he had a frank pulmonary hemorrhage which awakened him in the early morning. Bloody sputum continued for several days after this. One month later (December 3) the patient again expectorated bloody sputum. He went to a hospital and was given tincture of digitalis, but did not continue its use intelligently afterward. He did not recall having had rheumatism.

He suffered from diphtheria at the age of 4, for which he was given antitoxin. He had influenza at 14½ years.

On physical examination he was found to be suffering from auricular fibrillation. The temperature was 101° and continued to be slightly elevated for several weeks. It fell while he was taking digitalis. His heart was enlarged and a soft apical systolic murmur was heard. Occasionally a few râles were found on the extreme bases of the lungs after deep breathing. The blood pressure was 90-100 systolic and 60 diastolic. There was no edema. The urine was negative. The Wassermann reaction was negative. The urea index was 99. The kidney function was normal on the concentration and dilution tests and of the phenolsulphonethalein test (73.8 per cent). Under the influence of digitalis he made satisfactory progress, there was decrease in the ventricular rate, decrease in pulse deficit and alleviation of the symptoms of palpitation and dyspnea.

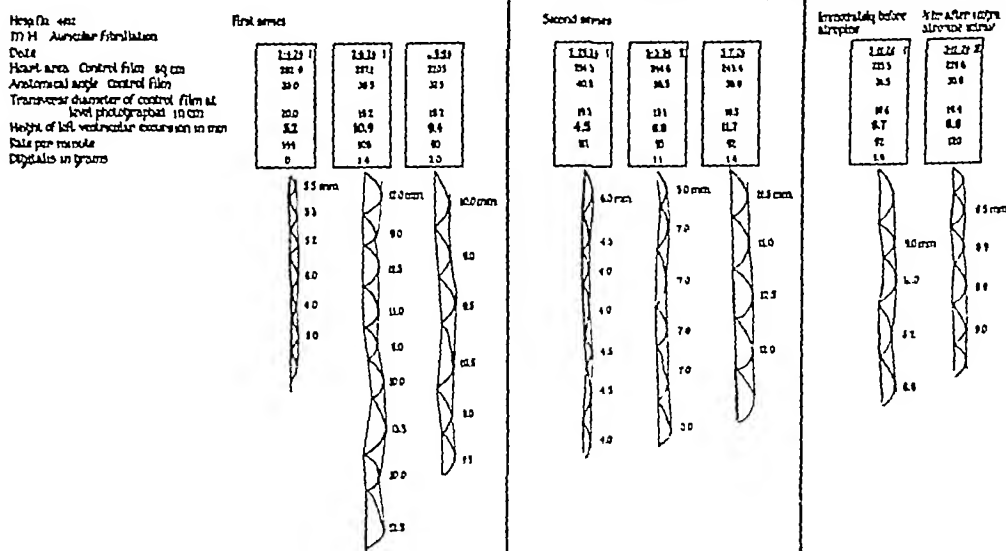


FIG 9 SEE FIGURE 5

On February 4, 1924, the excursion in the 5th interspace was 5.2 mm, the rate was 144 per minute. Two days later (February 6), after giving digitalis 1.4 gm, the excursion increased to 10.9 mm, the rate being 108 per minute (table 4, fig 9). On February 8, after a total of digitalis 2.0 gm had been administered the height of the excursion fell slightly to 9.4 mm, the rate was 90 per minute. The patient was given no more digitalis for 3 weeks, in the meantime the effect of the drug wore off. A second set of observations was then made. On February 29 (I), the excursion had fallen to 4.5 mm, the rate being 111 per minute. On March 3 (II) after digitalis 1.1

gm was given, the excursion increased to 6.8 mm, the rate being 95 per minute, and on March 7, after having taken digitalis 1.4 gm, it rose still farther to 11.7 mm, and the rate decreased slightly to 92 per minute.

In this patient, the effect of the ventricular rate on the extent of excursion was studied in greater detail. On February 4, when the rate was 144 per minute and on February 29, when it was 111, the patient was without digitalis, the ventricular excursions were 5.2 and 4.5 mm respectively. After giving digitalis the height of the excursion rose to 10.9 mm in the first series and to 11.7 mm in the second. The following test was then carried out on March 11, the height of the excursion was 8.7 mm and the rate, 92 per minute, the patient still being under the influence of digitalis. Immediately after this curve was taken the patient was given atropine 1 mgm intravenously, one half hour after the injection a moving film was again taken and the ventricular excursion measured 8.8 mm, the ventricular rate having now risen to 120 per minute. Although the heart rate increased 30 per cent, the height of the ventricular excursion remained unchanged.

The increase in rate did not influence the height of the excursion. It is of course not intended to infer that this statement applies in general. It applies only when digitalis has been administered. It is necessary that one of the two factors to be tested should remain constant.

This patient alone in the series showed a significant change in the size of the heart under the influence of digitalis. The decrease took place on the left side of the heart (table 4).

In order to show that the method is valid, the following measurements of a control normal individual (W. LaR.) over one year are given (table 5). On March 26, 1923, the left ventricular excursion measured 8 mm in the 4th interspace, the cardiac rate being 60 per minute. Ten days later the ventricular excursion measured 7.2 mm, and one year later, 8.1 mm, the heart rate remaining unchanged. These figures show a surprising constancy, they demonstrate the fact that comparable curves are obtainable not only over short, but also over long periods of time. Analysis of the control films showed that identical points on the left ventricular border had been photographed



TABLE 5  
*Measurements of curves of a normal subject at an interval of 1 year*

Date	Intercostal space* photo-graphed	Transverso diameter of control film at level photo- graphed	Analysis of moving film						Analysis of control film						
			Transverse diameter of original in inspiration		Transverse diameter of tracing in inspiration	Rate per minute	Left ventric- ular ex- cursion	Right auricular excursion	Area heart	Middle line to left border	Middle line to right border	Trans- verso diameter	Long diameter	Anatom- ical angle	
			of tracing in												
			Systole	Diastole											Systole
		cm	cm	cm	cm	mm	mm	sq cm	cm	cm	cm	cm	degrees		
3-26-23	11 S	12.7	13.2	11.2	13.0	11.0	60	8.0	5.2	129.7	8.6	1.5	13.1	11.8	39.5
1- 5-23	41 S	13.1	12.7	13.7	12.6	11.0	60	7.2	4.0†	133.1	8.8	4.1	13.2	15.1	10.5
3-12-24	11 S	12.8	13.0	14.1	13.0	11.1	60	8.1	1.7	131.8	8.3	4.7	13.0	15.6	46.5

\* Superposition of films demonstrated that identical points of the left ventricle were actually photographed

† Right auricular shadow not sharp

on the moving film and that the heart had not changed in size during the period of the observation

#### DISCUSSION

What part slowing the heart rate may play in increasing the ventricular excursion after giving digitalis has already been indicated. It has been shown that for the rates within the range we met in our observations this factor is negligible. Beyond certain narrow limits however other influences obviously come into play, and may naturally be expected to change the height of the excursion. What effect change in the height of the blood pressure may have on the extent of the ventricular excursions remains to be discussed.

It was discovered in 1839 by Blake that giving digitalis to animals under experimental conditions causes an elevation of blood pressure. This effect is now well known. In more recent years Gottlieb (1901, 1902) and his co-workers have associated this effect with constriction of the blood vessels, due to a direct action of digitalis on the vessel walls. However this may be, the problem of its action in man is concerned not so much with local effects on the vessels in distant viscera, such as the kidneys and intestines, which have been studied by Jonescu and Loewi (1908), Kasztan (1910), Hedinger, (1912) Naegele, (1911) Fahrenkamp (1911) and Joseph (1913), as with the effect on aortic pressure which results from this action. For it is against aortic pressure that the heart must work. Joseph (1913) states that in rabbits it requires only 28 mg per kilo to increase contraction 12 per cent, but 50 mg to bring about even a slight rise in blood pressure. Experiments dealing with this phase of the matter have recently been carried on by de Heer (1912), Straub (1914), Patterson, Piper and Starling (1914), and Wiggers and Katz (1922). Their experiments have shown that, although the systolic and diastolic cardiac volumes are both increased by raising the resistance offered by the aorta, the *extent* of the excursion in contraction is *unchanged* until the resistance to be overcome passes a certain limit, and then there is a decrease in the height of the excursion. Even if giving digitalis raises the aortic pressure, it is unnecessary therefore to expect, in the light of these experiments, a change in the excursion of the ventricular wall. In the case of our observations, the diastolic

volumes, measured in terms of the transverse diameter and the areas of the cardiac silhouette, did not even change, in one case, indeed, they actually decreased

It was usually believed on the basis of experiments in pharmacology, before 1900, when taking the blood pressure in human beings was still uncommon, that giving digitalis raised the pressure. This belief was gradually abandoned after the statements on this subject by Gottlieb and Sahli (1901). Since the more extended observations by Mackenzie (1911), Cushny (1911), Cohn (1917), Robinson (1920), Eggleston (1917) and Luten (1924) the fact is now established that no change in the systolic blood pressure takes place under the influence of digitalis, while the diastolic pressure, unless heart failure is present, often falls. When heart failure is present the elevated systolic pressure usually falls as well.

In the four patients included in this study, no definite changes in the resting blood pressure levels were observed, when they were under the influence of digitalis. The systolic level remained unchanged four times (Case 1, Case 3, twice, Case 4), decreased 16 mm Hg once (Case 2), and increased 10 mm Hg once (Case 4). The diastolic level was unchanged three times (Case 1, Case 4, Case 3), and decreased 10 mm Hg twice (Case 4, Case 2). In none of the observations was a *rise* in the diastolic pressure seen.

There were seen, in short, no significant or consistent changes in either the systolic or diastolic blood pressure. The changes in the excursion of the ventricle which we saw, were, therefore, not influenced by this as an important factor. Nor are the slight changes, assuming that they took place, of such a nature as to be able to bring about the effects we noticed.

#### SUMMARY

The effect of digitalis in therapeutic doses was studied in patients by the method of the moving x-ray film. Four patients were studied in this manner. Two of these exhibited a normal rhythm and the other two that of auricular fibrillation. In all cases a significant increase in the height of the left ventricular excursion was seen when the patients were under the influence of digitalis. As the effect of the drug wore off the height of the excursion decreased. As a rule



no decrease in the size of the heart took place We believe that this is the first time that the effect of a drug upon the contraction of heart muscle in man has been studied directly

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# EXPERIMENTAL STUDIES ON RAPID BREATHING

## I TACHYPNEA, INDEPENDENT OF ANOXEMIA, RESULTING FROM MULTIPLE EMBOLI IN THE PULMONARY ARTERIOLES AND CAPILLARIES

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### INTRODUCTION

Rapid breathing is often a striking phenomenon in diseases of the cardio-respiratory systems, particularly in passive congestion of the lungs associated with heart failure and, more so, in lobar pneumonia. Indeed, in pneumonia accelerated respirations may be the outstanding clinical feature of the disease and are often a sign of prognostic importance. No physician likes to see a sudden increase in the respiratory rate or a continuation of rapid respirations over a prolonged period. But the causes of tachypnea are by no means clear nor are its effects understood. Haldane and his co-workers (1) have taught that rapid and shallow breathing may lead to anoxemia which, in turn, tends to keep up the condition of rapid and shallow breathing, thus establishing a vicious cycle. Meakins (2) has presented evidence for the view that increased respiratory rate and decreased depth, as observed in pneumonia, may be in part responsible for the occurrence of the anoxemia so frequently seen in this disease. At what point the vicious cycle begins has not been fully understood, but according to these investigators, in such conditions as pneumonia, anoxemia conceivably arises from the unequal distribution of the air in the lungs. This initiates rapid and shallow breathing, thus causing severe anoxemia and, in turn, more rapid and more shallow respirations.

It has seemed to us important to inquire further into the nature of this mechanism and to investigate experimentally some of the causes and some of the effects of tachypnea. We have already ap-

proached this problem in its relation to lobar pneumonia from several points of view. A study (3) was made of the fluctuations in lung volume throughout the course of the disease. It was found that the volume of air in the lungs varied coincidentally with the clinical course of the disease, but no unequivocal correlation could be established between lung volume changes and variations in the rate and depth of respirations. A more recent study (4) of the acid-base equilibrium of the blood of patients suffering from lobar pneumonia revealed no changes which could be regarded as responsible for these abnormal respiratory phenomena.

Since neither the gross volume changes alone in the lungs, nor the chemical changes in the blood presented explanations for the functional changes in which we were interested, it seemed to us important to investigate the nervous factors concerned with the control of the respiratory rate.

From a consideration of the phenomena involved in the control of normal respiratory movements, it is apparent that certain reflex impulses, probably arising in the lungs, may be responsible for changes in the rate and depth of the respiratory excursion, and thus, under abnormal conditions, provide a means for the onset of rapid and shallow breathing. Normally, a self-regulating mechanism exists (the Hering-Breuer (5) reflex) whereby, through the function of the vagus nerves, a given respiratory phase is terminated and the reverse phase initiated. Since the description of this reflex by Hering and Breuer, it has been generally taught that the alternate distension and collapse of the lungs provide the stimulus for limiting one respiratory phase and releasing the opposite. The true nature of the stimulus is not understood, but it is probably in some manner related to the alternate stretching and slackening of the alveolar walls during inspiration and expiration. Lumsden (6) has brought some evidence in favor of the reflex being stimulated by the alternate inrush and outrush of air over the ciliated epithelium of the trachea and bronchi. The cause of the rhythmic discharge of the respiratory center is equally as obscure. Indeed, 34 years ago Henry Head (7) made the following statement which still must be regarded as substantially true:

If we attempt to take a general survey of the nervous mechanism of respiration, we must begin by confessing that we are entirely ignorant of the cause of the

rhythmic activity of the respiratory centre Although the vagi play an important part in regulating the breathing they certainly are not the ultimate cause of rhythmic respiration, for rhythmic breathing still continues, although in an altered form, even after the vagi have been divided Moreover the centre still sends out rhythmic impulses even when the medulla oblongata is separated from the rest of the brain, the spinal cord severed below the seventh cervical vertebra and the vagi, superior laryngeal, and glosso-pharyngeal nerves divided Now whatever may be the stimulus which keeps up the activity of the respiratory centre it is certainly not of a rhythmic nature, and we are brought face to face with the difficulty that a continuous stimulus produces discontinuous activity in the organ upon which it acts So far we are unable satisfactorily to explain why this should be, but it is one of the earliest phenomena which meet us in the study of vital activity

In the absence of any precise knowledge as to the nature of the rhythmic impulses normally arising in the respiratory centre or of the character of the reflex impulses arising in the lungs, it is unlikely that we can arrive at any complete understanding of the disturbances of respiratory rhythm On the other hand, a certain body of evidence exists for the belief that the afferent impulses, arising from local stimulation of the vagus nerve endings located in the lungs, may possibly account for the rapid and shallow breathing observed in such conditions as acute lobar pneumonia Porter and Newburgh (8) observed that the dyspnea which accompanied experimental pneumonia in dogs produced by Friedlander's bacillus could be checked by vagotomy or prevented by section of the vagi previous to infection They concluded from their experimental findings that blocking of the afferent vagal impulses saved the respiratory centre from fatigue Other investigators have reported that local stimuli, due to irritants, (Pi Süner (9)), or to carbon dioxide, (Scott (10)), (Pi Süner and Bellido (11)), may bring about rapid respirations which are promptly stopped by section of the vagus nerves This phase of the problem received renewed attention during the war when the curious disturbances of respirations present in gassed soldiers and in those suffering from the so-called "effort syndrome" were observed Haldane (12) interpreted these pathological states as due to changes in the excitability of the respiratory center rather than to alterations in the threshold of activity of the Hering-Breuer reflex In an effort to analyze the factors involved in the tachypnea due to gassing, Dunn (13) made the



striking observation that obstruction to the pulmonary circulation in goats, brought about by the intravenous injection of a suspension of potato starch, gives rise to a pronounced increase in the respiratory rates, unassociated with the appearance of arterial or venous anoxemia. Furthermore, he observed that vagal section prevented the onset of rapid breathing, or abolished it when it had already begun. In spite of carefully planned experiments to estimate the blood flow, arterial and venous blood pressure changes, etc., Dunn did not explain the cause of the rapid respirations which intravenous starch injections initiated, but believed them to be in some way related to spasm of the finer bronchial musculature.

It follows from the foregoing discussion that further investigation of peripheral afferent stimuli in relation to rapid breathing is necessary. This problem was the incentive for our own experiments, of which an account follows.

#### EXPERIMENTAL

*Choice of anesthetic* Experiments on respiratory control in man, as well as in the lower animals, are complicated by voluntary, emotional, and reflex influences. To obviate these in lower animals it is usually necessary to resort to the use of an anesthetic, the choice of which is of signal importance. Depression of the respiratory centre, or inhibition of peripheral reflexes due to the anesthetic, may so alter the mechanism as to lead to false interpretations. Ordinary volatile anesthetics, such as ether and chloroform, cannot be used satisfactorily in respiratory studies. Gad (14), following the observation of Guttman (15) recommended the use of chloral hydrate. Though possibly efficient for rabbits, chloral hydrate does not wholly satisfy the requirements when dogs are being used. The effect is not sufficiently uniform or lasting. After repeated preliminary trials it was found that Luminal and Luminal Sodium (Winthrop) provided almost ideal conditions. It is used in dosages of 0.12 to 0.15 gram per kilogram given by stomach tube. Complete narcosis does not appear for 4 or 5 hours. Anesthesia is then so light and even, that a corneal reflex usually persists throughout the experiment. Breathing remains quiet and regular, usually rather slow. The arterial blood is usually about 90 per cent saturated with oxygen. The dogs retain their sensitiveness to intratracheal and intrapulmonary stimuli. For example, a fine catheter passed through the trachea into one of the smaller bronchi leading to a lobe elicits an expulsive reflex associated with changes in respiratory rate. This reflex is often completely abolished with other anesthetics, but its persistence is of importance in the study of the effects of peripheral stimuli arising in the lungs. After several preliminary experiments in which rapid breathing was induced by irritant substances (chlorine water,  $\text{NH}_4\text{OH}$ )

we determined to repeat Dunn's experiments for the purpose of ascertaining whether obstruction to the pulmonary circulation (arterioles and capillaries) in dogs gives rise to rapid breathing

*Embolism of the pulmonary circulation produced in dogs by the intravenous injection of a suspension of potato starch*

In attempting to repeat Dunn's experiments on dogs, instead of goats which he used, we were at first confronted with the appearance of sudden death before any changes in the rate of respirations occurred. We learned, however, that this could be prevented by altering the method of starch injection. It was necessary to keep the starch granules from settling by bubbling a fine stream of air through the salt solution in which they were suspended. The suspension was then allowed to run slowly and intermittently from a burette into the right external jugular vein which had been cannulated with a wide bore glass cannula.

The starch suspension used was made according to Dunn's directions by scraping a peeled potato on a grater, washing in 0.85 per cent NaCl solution, filtering through 6 to 8 layers of gauze, and allowing the granules to settle. The sediment was measured in a graduated cylinder and 3 times its volume of 0.85 per cent NaCl solution was added. Later in these experiments it was found of advantage to use a more dilute suspension—1 part of starch to 19 parts of physiological salt solution.

Under these conditions it was observed that a certain volume of starch suspension could be injected—without producing any apparent effect. Further injection, however, resulted in very definite and constant changes. The first to be observed was an increase in respiratory rate. Associated therewith was a modification of the character of the respiratory movements, the maximum excursion of the body wall shifting from the thorax to the region of the diaphragm. During the expiratory phase the abdominal muscles at the level of the diaphragm appeared to contract forcibly. The rate gradually accelerated and the depth appeared to grow shallower. At this point further starch injection almost invariably killed the dogs. Acceleration continued until the breathing became very rapid (often more than 100 to the minute) and apparently very shallow. During the period of rapid breathing the tongue and mucous membranes and the pads of the

paws were usually bluish and dusky After several hours of this condition respiration gradually slowed and then ceased, the heart continuing to beat for some minutes before the death of the animal

This description may be regarded as typical of a starch experiment It presented several facts for quantitative analysis

(a) *The relation of rapid breathing to the volume of starch suspension injected* No constant dosage could be found which would surely bring on tachypnea Some dogs responded to one injection of 5 cc of the 1 4 suspension, others required from 15 to 20 cc With the more dilute suspension, 1 20, larger volumes were necessary, 40 cc in one experiment

The fact that a certain volume of starch suspension could be injected without effect on respiratory rate was shown in an experiment in which the rate remained at 16 per minute even after a total of 11 cc of a 1 4 suspension had been injected Not until a total injection of 15 cc had been given did acceleration begin The characteristic acceleration following starch embolism is seen in another experiment where, after a total of 10 cc of 1 20 starch suspension the rate remained at 12, but after a total of 40 cc the rate gradually accelerated from 12 to 100 breaths per minute in the hour following injection The data of these experiments are presented in table 1

These facts must be regarded as significant They suggested to us that the starch effect was probably not an irritative one, involving principally afferent impulses which might be expected to operate at once in small doses, but that the effect was related in some way to the quantitative obstruction of the pulmonary circulation

(b) *Changes in pulmonary ventilation following starch embolism* It has already been mentioned that the rapid respirations following starch injection had the appearance of being shallow This point was definitely established by connecting a pair of flutter valves to the tracheotomy tube and collecting the animal's expired air in a Tissot spirometer in which the volume of air could be measured In one such experiment the tidal air was 147 cc with a rate of 14 and a minute volume of 2 06 liters before starch injection, as contrasted with a tidal air of 88 cc, a rate of 50 and a minute volume of 4 40 liters after starch injection This is characteristic of the rapid and shallow breathing seen in disease in man in which the combined effect of

increased rate and decreased depth leads to a greater minute volume of pulmonary ventilation, but because of the relatively larger dead air space to a decrease in effective alveolar ventilation

TABLE 1  
*The effect of starch injection on respiratory rate*

Experiment number	Time	Total starch	Respiratory rate per minute
		cc.	
17	4 29	0	16
	4 42	8 4	16
	4 53	11 0	16
	4 58	15 0	24
	5 15	17 0	28
	5 30		38
21	11 25	0	12
	12 09	10 0	12
	12 10 to 12 25	40 0	
	12 26		24
	12 29		30
	12 33		36
	12 36		40
	12 45		46
	12 55		54
	1 10		66
	1 16		100

TABLE 2  
*Intravenous injection of potato starch*

Experiment number	Time	Total starch suspension	Respiratory rate per minute	Arterial blood			
				O <sub>2</sub> content	O <sub>2</sub> capacity	Per cent saturation	CO <sub>2</sub> content
		cc.		vol. per cent	vol. per cent		vol. per cent
17	4 29	0	16	14 47	16 63	87 6	38 85
	6 29	22	54	16 76	20 24	82 8	35 85
	6 47		86	15 33	21 17*	72 4	30 67
19	2 40	0	18	16 71	17 75	94 2	47 30
	3 33	5	53	13 10	17 57	74 5	52 00
	3 47		73	10 99	16 48	66 7	51 60

\* The increased O<sub>2</sub> capacity observed here is probably associated with concentration of the blood resulting in part from pulmonary edema.

(c) *Arterial anoxemia following starch embolism* The cyanosis of the tongue and mucous membranes which we observed after the onset of rapid and shallow breathing was not mentioned by Dunn in his experiments. Nor did he find a condition of anoxemia of the arterial blood. The occurrence of cyanosis in our experiments indicated the probable existence of arterial anoxemia. This we found to be true. In four starch injection experiments the average arterial  $O_2$  content before injection was 14.73 volumes per cent as compared with 11.74 volumes per cent after embolism, the average  $O_2$  capacity being 16.49 volumes per cent before and 16.31 volumes per cent after embolism. These changes resulted in an average percentage saturation of 89.3 before embolism and 71.6 after. The detailed findings in two of these experiments are shown in table 2.

(d) *Effect of oxygen inhalation on anoxemia and tachypnea resulting from starch embolism* To determine the relation between rapid and shallow breathing and anoxemia the following experiment was performed.

*Experiment 21* A dog weighing 10.5 kg. was given 1.65 grams Luminal by stomach tube. Three and one-quarter hours later the animal was relaxed and anesthetized. A tracheotomy was done, the right external jugular vein cannulated for starch injection, and the left femoral artery was cannulated for the purpose of drawing samples of blood for gas analysis. At 3.13 p.m. with the dog breathing room air, his respirations were 14 to the minute and his arterial blood was normally oxygenated, the percentage saturation being 92.8. One hour and forty-seven minutes later, after the dog had received intravenously 14.5 cc. of 1.4 starch suspension, his breathing had accelerated to 89 to the minute, he was cyanotic, and his arterial blood was 77.5 per cent saturated. The dog was then permitted to breathe 90 per cent oxygen. This resulted in a disappearance of the cyanosis. The arterial blood was no longer unsaturated but showed a percentage saturation of 96.8. In spite of this fact, his respirations had accelerated to 99 to the minute. On discontinuing the oxygen supply and allowing the dog to breathe room air once more, cyanosis became intense, the percentage saturation of arterial blood falling to 55.6, and the respirations growing extremely rapid and shallow—194 to the minute.

We believe that this experiment indicates that anoxemia is not primarily responsible for the occurrence of the rapid and shallow breathing of starch embolism. For here the rate continued at 99 to the minute without anoxemia though, to be sure, the further acceleration to 194 was undoubtedly the result of oxygen want.

In another experiment this same point was brought out by a reduction in rate from 74 to 47, upon breathing 90 per cent oxygen—the rate before embolism having been 18. This reduction was associated with disappearance of anoxemia, but it is apparent that the primary cause of tachypnea which produced a rate of 47 still operated.

More convincing evidence of the fact that tachypnea following starch embolism occurs independently of anoxemia was furnished by an experiment in which a dog inhaled 83 per cent  $O_2$  and maintained completely saturated arterial blood throughout. In spite of this, the rate of his respirations rose from 14 to 50 after 9.5 cc of 1:20 starch suspension had been injected intravenously.

These observations are consistent with the findings of Dunn, who observed in goats rapid breathing without anoxemia.

The cause of anoxemia following obstruction to the pulmonary circulation is fully discussed in Paper II of this series (16). It may be momentarily dismissed here since it has been shown that the rapid breathing in which we are primarily interested may occur independently of anoxemia. It remains, therefore, to inquire into the cause of tachypnea. In the introduction we have already considered the probable relation of rapid and shallow breathing to the reflex innervation of the lungs. As long ago as 1812 Legallois (17) observed that section of the vagus nerves produced slowing and deepening of the respiration. Hering and Breuer (5) as well as Head (7) showed that the reflex mechanism, which goes by their names, depends for its existence upon the function of the vagi. Gad (14) was the first to demonstrate that by freezing the vagus nerves their functional activity could be temporarily interrupted, subsequently to be restored by thawing. This method of physiological vagotomy has the twofold advantage over actual section of the nerves in that it eliminates complicating currents of injury induced at the cut ends of the nerves which, of themselves, alter the type of breathing, and in that subsequent thawing permits restoration of normal conduction.

#### *Method of vagal freezing*

A convenient method for freezing the vagi is to place under the isolated intact nerves a silver plated tube 2 mm in diameter, so bent as to allow the nerves to lie in two concave depressions, with the dog's trachea disposed between them.

(see fig 1) The tissues, other than the vagi, are insulated from the tube by cotton wool. Through this tube cold brine is permitted to flow, the temperature of which can be controlled by the proper admixture of a warmer brine solution

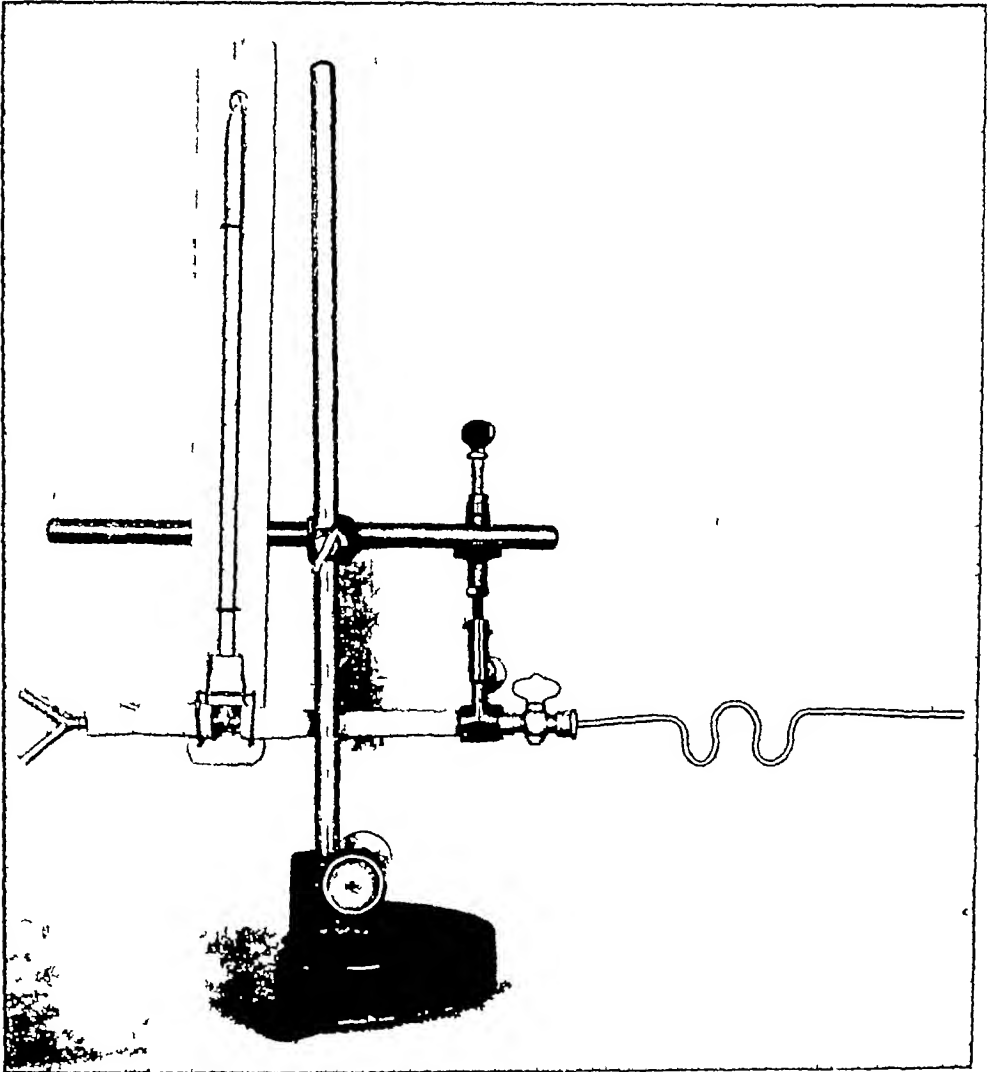


FIG 1 PHOTOGRAPH OF BENT SILVER PLATED TUBE THROUGH WHICH COLD BRINE FLOWS—FOR THE PURPOSE OF FREEZING THE VAGUS NERVES

The optimum temperature for freezing lies between  $0^{\circ}$  and  $-5^{\circ}\text{C}$ . At these temperatures subsequent thawing apparently restores the nerves to normal functional activity.

*Effect of vagal freezing upon tachypnea following starch embolism*

*Experiment 60* A dog anesthetized with Luminal Sodium was given 9.5 cc. of 1:20 starch suspension intravenously. His respiratory rate rose from 16 to 57 per minute. While breathing at this rate the isolated vagi were frozen by the method described, with the result that the rate of breathing immediately dropped to 20. The accompanying changes in tidal air were as follows: Before embolism 147 cc., after embolism 88 cc., after vagal freezing 170 cc.

The effect of this procedure is, therefore, to convert rapid, shallow breathing into slow, deep breathing. We might conclude from such an experiment that the physiological section of the nerves occasioned by freezing them blocked certain afferent peripheral impulses initiated by the presence of the starch emboli. We have, however, already cited evidence which casts doubt on the starch effect being primarily the result of afferent irritative impulses. It was shown that a certain mass of starch suspension had to be injected before tachypnea was precipitated. Unless we are dealing with a summation of inadequate stimuli this fact strongly suggests that the starch effect is a mechanical one, rather than irritative, resulting from obstruction to a certain portion of the pulmonary circulation—or, at least, from the anatomical changes secondary to such obstruction.

*Effect of vagal freezing upon tachypnea due to central rather than peripheral stimuli* In order to find out whether the immediate subsidence of tachypnea brought about by vagal freezing after starch embolism necessarily indicated that the tachypnea had been due to afferent peripheral stimuli arising in the lung, a control experiment was planned in which rapid breathing was induced by central stimulation (anoxic anoxemia, inhalation of 10 per cent  $\text{CO}_2$ ). In this experiment there was no question of abnormal peripheral stimuli such as might result from starch embolism.

*Experiment 63* A dog weighing 12.5 kg. was given 1.9 gram Luminal Sodium by stomach tube. Two and one-quarter hours later when he was relaxed and anesthetized, a tracheotomy was performed and the left femoral artery was cannulated. The vagus nerves were exposed in the neck and placed on the freezing tube. The dog was then made to rebreathe a certain volume of air enclosed in a Benedict spirometer, equipped with inflow and outflow valves, the  $\text{CO}_2$  being continuously removed by passage of the expired air through soda lime.



The result of this procedure was the gradual utilization of the oxygen in the spirometer until the animal developed oxygen want and consequent rapid breathing. At the height of rapid breathing, when the  $O_2$  concentration in the spirometer had fallen to 3.9 per cent, and the animal was deeply cyanosed, the vagi were frozen. This resulted in an immediate slowing and deepening of respirations. These facts were graphically recorded by the spirometer and are repro-

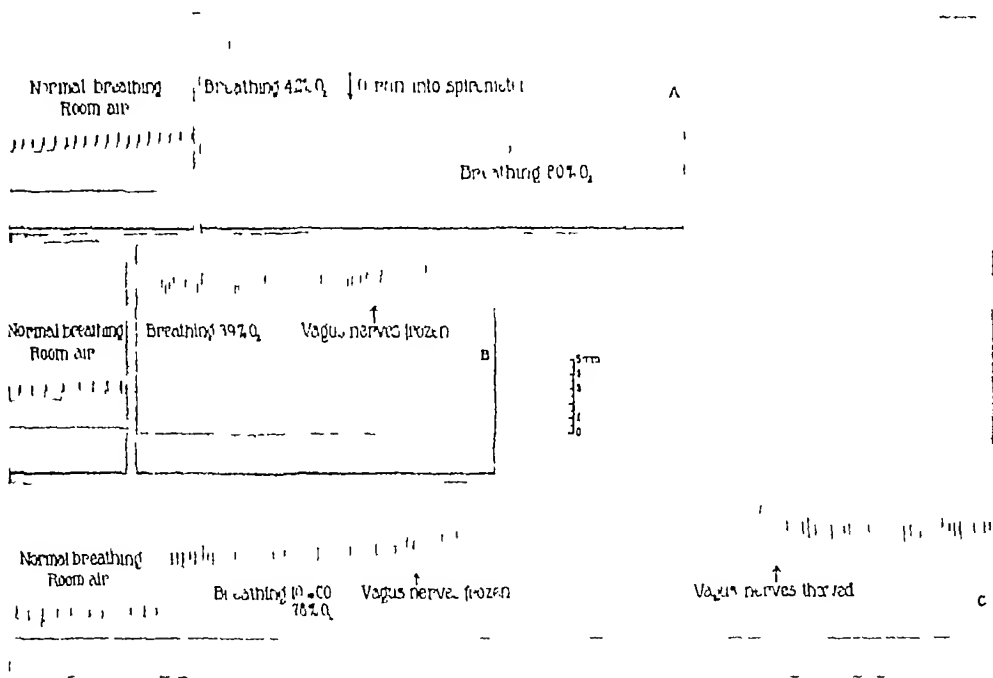


FIG. 2 THE EFFECTS ON RESPIRATION OF A LOW  $O_2$  CONCENTRATION, VAGAL FREEZING AND HIGH  $CO_2$  CONCENTRATION

Curve A shows the slowing effect on respiratory rate of adding 90 per cent  $O_2$  to the oxygen poor mixture in the spirometer. Time marker indicates 1 second intervals.

Curve B shows the slowing and deepening effect of vagal freezing on rapid respirations due to breathing an oxygen poor mixture. Time marker indicates 1 second intervals.

Curve C shows the slowing and deepening effect of vagal freezing on rapid breathing due to high  $CO_2$  concentration. Time marker indicates 5 second intervals. The factor for the spirometer is 4.82 cm excursion of the bell for 1 liter. The scale represents centimeters reduced proportionately to the tracing.

duced in figure 2, curve B. To show that the rapid respirations were due wholly to oxygen want in another such period of rebreathing when the  $O_2$  concentration of the spirometer had fallen to 4.2 per cent, 95 per cent oxygen was run into the spirometer with the result that rapid respirations immediately ceased. This is graphically shown in figure 2, curve A.

This experiment, therefore, showed that rapid breathing due to oxygen want resulting from lowered alveolar oxygen tension can be at once stopped by freezing the vagus nerves. To find out whether vagal freezing checks the rapid breathing resulting from central stimuli other than anoxemia, the same dog was permitted to rebreathe a volume of 95 per cent oxygen enclosed in the Benedict spirometer from which the soda lime had been removed. The result was a gradual accumulation of  $\text{CO}_2$  without the development of  $\text{O}_2$  want because of the high  $\text{O}_2$  concentration. When the  $\text{CO}_2$  concentration had reached 10 per cent and the dog's breathing was rapid, the vagi were frozen and there resulted slow, deep respirations. The graphic spirometric tracing is reproduced in figure 2, curve C.

This is in accord with the findings of Scott (10), who showed that the response to high  $\text{CO}_2$  inhalation after vagotomy was characterized by increase in depth rather than accelerated rate.

We believe that this experiment shows that since vagotomy slows the tachypnea of central origin, such slowing does not necessarily imply the blocking of afferent irritative peripheral impulses. And the slowing produced by vagal freezing in starch tachypnea cannot, therefore, be used as evidence for the existence of such impulses.

#### PATHOLOGY OF STARCH EMBOLISM

At this point a description of the pathological process produced in the lungs by starch embolism will be of advantage. It should be said that the potato starch granules are of variable shape and size, being, roughly circular to oval, and in diameter from 20 to 40 micra. In a starch suspension some granules were seen with a diameter as small as 5 micra and others as large as 60 micra. The diameter is such as to permit their entrance into terminal arterioles and capillaries, but the granules are apparently too large to pass beyond pulmonary capillaries. Starch cells have not been found on histological examination of organs other than the lungs. But in the lungs their distribution is widely disseminated. In some specimens they are frequently seen in almost every low power microscopic field, often completely obliterating the lumen of a capillary. A detailed description of the gross and microscopic pathology of the embolized lungs follows.

*Pathology of starch lungs* Postmortem examination was performed on 15 dogs which had received starch. In some instances the animals were killed at the conclusion of the experiment by injecting 10 to 20 cc of a saturated solution of magnesium sulphate<sup>1</sup> intravenously, in other cases they died spontaneously. Autopsy was performed immediately after death, the trachea being clamped before the chest was opened. The clamp was later removed and the collapse of the lungs noted.

*Normal controls* Two normal dogs were sacrificed to serve as controls. These were anesthetized and fastened to the table for 3 hours, in a way comparable to the experimental animals. One of these died following decerebration, and the other was killed with magnesium sulphate intravenously.

The lung-heart ratio in the 2 normal controls was 1.42 and 1.10 respectively, the mean being 1.26. The pleural cavities contained no free fluid. The lungs were pale pink and collapsed completely on removing the tracheal clamp, except in small areas in the cephalic and ventral lobes<sup>2</sup>. Only a slight degree of hypostasis occurred in the posterior of the caudal lobes of these animals. The right heart seemed moderately dilated.

### *Gross pathology*

For convenience of description the material may be divided into two groups according to whether the animals died, or were killed, within 2 hours after the primary starch injection, or longer, 2½ to 4½ hours. Eight animals are included in the former, and 6 in the latter group.

*Thorax and lungs* In the first group, i.e., in those dogs in which death occurred within 2 hours after the first starch injection, the lungs appeared normal except for more extensive hypostasis in the dependent parts. There was no marked congestion and no gross edema. The heart-lung ratio in 2 cases was 1.44 and 1.45, respectively, which is within the normal limit. No free fluid was present in the pleural cavities.

In the second group of 6 animals, in which death occurred from 2½ to 4½ hours after the first starch injection, pleural fluid was present in excess in only 1 (25 cc), but hypostasis was more extensive than in the first group in all. In 1 dog hypostasis was so extensive as to involve the whole of both caudal lobes and the dependent (dorsal) parts of the other lobes. In all the animals of this group the

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<sup>1</sup> For this method of killing dogs we are indebted to Colonel E. B. Vedder of the United States Medical Corps, Medical Research Division, Chemical Warfare Service. The method has the advantage of bringing about almost instantaneous death from respiratory failure and cardiac stand-still, without resulting structural changes in the lung attendant upon most other methods of killing animals.

<sup>2</sup> For convenience Theobald Smith's (18) classification of the lobes of the lungs is employed. On either side there is a cephalic ventral and caudal lobe, while on the right side there is, besides, a medial lobe.

lungs collapsed on removing the tracheal clamp, but not to the same degree as in the normal dogs. There was, however, no generalized emphysema. Petechial hemorrhages occurred on the pleural and on the cut surfaces of the lungs in 4 instances. The hemorrhagic areas were bright red and measured approximately 3 to 4 mm in diameter. Edema was very marked in the lungs of 2 dogs, frothy serous fluid exuding in large quantity through the trachea when the lungs were inverted, and the cut surfaces appearing quite wet. The lung-heart ratio in these 2 was 4.63 and 3.85, respectively, well over the normal figure, and in 1 other it was 1.66.

*Other organs* The right heart usually appeared dilated and tense. The spleen, liver and kidneys showed no lesions which could be attributed to starch injection.

*Antemortem injection of India ink* In 1 case 25 cc of Higgin's waterproof India ink, previously dialyzed in a parchment sac against Ringer's solution (Krogh (19)), was run into the jugular vein and the animal killed 5 minutes later by the intravenous injection of magnesium sulphate solution. Examination of these lungs after removal showed that the hypostatic areas in the lower lobe were red and had not been permeated by India ink. The remainder of the surface of the lungs was stippled with small black spots—a picture very different from that seen in a normal animal similarly injected with India ink. In a normal dog the surface of the lungs presented a diffuse black discoloration except along the edges where there was no India ink to be seen.

*Postmortem injection of barium sulphate gelatin* The pulmonary arteries of 2 normal dogs and of 2 "starch" animals were injected postmortem by the method of Gross (20) with barium sulphate gelatin under 40 to 60 mm Hg pressure. The solution used, however, was less viscous than Gross's having as its base 6 per cent gelatin, which is approximately isoviscous with blood. After fixation in 10 per cent formalin, stereoscopic x-ray photographs were made. The lungs were then cleared by the Spalteholz (21) method.

Examination of stereoscopic x-rays of the injected lungs of the normal dogs shows that the main arteries gradually diminish in calibre as they approach the periphery, and that they give off numerous small branches which similarly diminish in size to end in the fine arterioles at the surface. The structure may be compared to the branching of a spruce tree (see fig. 3). In the x-ray pictures of the starch lungs careful examination shows that the shadow cast by the fine thread-like terminal vessels is absent. This is demonstrated more clearly by inspection of the surface of the cleared specimens with a lens (see figs. 4 and 5). There is also a noticeable difference in color between the cleared normal and starch specimens. The starch lungs are much darker than the normals, due to retained blood which has not been completely washed out by the preliminary saline irrigation.

*Microscopic pathology*

*Lungs* (See fig 6) Histological examination was made in 13 cases After the lungs had collapsed sections from several lobes were fixed in Zenker's fluid with 5 per cent acetic acid Eosin and methylene blue staining was used on



FIG 3 DOG 6 STARCH INJECTION X-RAY PHOTOGRAPH OF CEPHALIC, VENTRAL AND MEDIAL LOBES OF RIGHT LUNG AFTER INJECTION WITH BARIUM SULPHATE GELATINS

*Note the type of branching of the pulmonary artery in the dog's lung*

paraffin embedded sections In some instances frozen sections were stained with Gram's iodine

The starch granules were seen in the arterioles and capillaries scattered throughout all lobes, the majority appearing to be in arterioles Those in the capillaries



FIG 4 DOG C 1 NORMAL CONTROL NATURAL SIZE PHOTOGRAPH OF SURFACE OF INJECTED AND CLEARED RIGHT LUNG

*Note the fine peripheral vessels* The animal was bled to death and the right pulmonary artery irrigated with saline at 30 to 40 mm Hg pressure until the return venous flow was colorless. It was then injected with hot 6 per cent barium sulphate gelatine at 50 to 60 mm Hg pressure and fixed in formalin, dehydrated and cleared in oil of wintergreen.



FIG 5 DOG 16 NATURAL SIZE PHOTOGRAPH OF "STARCH" LUNG

*Note the absence of the finer arteriole tufts—and the dark color due to retained blood*

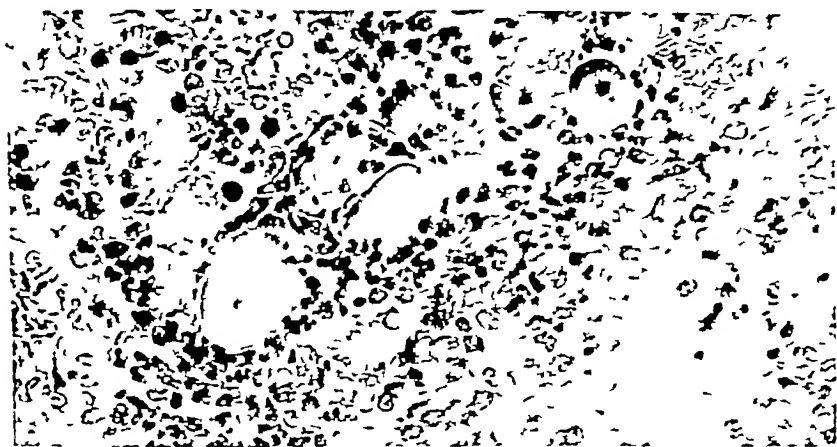
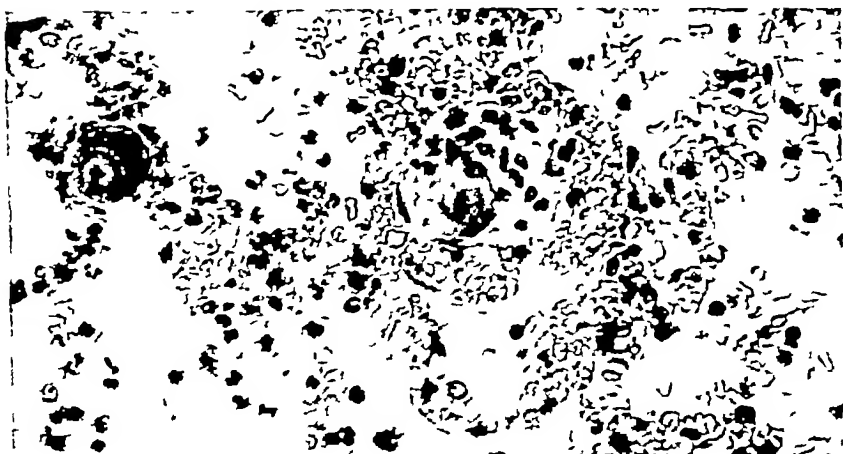


FIG 6 DOG 21 "STARCH" LUNG MICROPHOTOGRAPH OF RIGHT VENTRAL LOBE  $\times 430$

Grossly the lung showed petechial hemorrhages and edema

A Note the two starch granules with surrounding leucocytic thrombi. The great local dilatation of the capillaries is also evident

B The later stage of the above. Note the extravasation of red blood cells into the alveolar lumina which also contain a serous fluid and some leucocytes



caused definite dilatation of the walls and bulging into the alveolar lumina. The number of granules varied greatly in different specimens, sometimes occurring in every low power field, sometimes not so frequently. Frequently small leucocytic thrombi could be seen about the granules. An interesting finding was the irregular narrowing of the lumina of the bronchioles, due to local thickening of the musculature. This was likewise observed by Dunn who believed it represented muscular spasm. The significance of these contractions is doubtful, since they were found also in the lungs of the normal dogs.

In the 7 dogs in which death occurred within 2 hours after the first starch injection the characteristic findings were those just described. Besides these, in 4 instances areas of congestion and partial atelectasis were seen about the starch emboli. In these congested areas capillaries were distended and the alveolar walls thickened. Interstitial edema occurred in 2 of these 4 dogs and in 1 other of this group.

On the other hand, in each of the 6 cases in which death occurred  $2\frac{1}{2}$  to  $4\frac{1}{2}$  hours after the first starch injection, interstitial edema was present, especially in the loose tissue surrounding the blood vessels. In 4 of these animals there was, as well as interstitial edema, congestion and dilatation of the capillaries in the region of the starch emboli with extravasation of fluid and red blood cells into the alveolar spaces. The walls thus thickened encroached on the alveolar lumina. Emphysema did not occur except in small areas near the surface.

The above description does not include sections of the hypostatic areas which showed hemorrhagic extravasation into the alveolar spaces.

### *Blood pressure following starch embolism*

From the obstruction to the pulmonary circulation observed in these pathological specimens it seemed at first probable that starch embolism might lead to profound changes in systemic and pulmonary blood pressures and that the rapid breathing following starch injection might be related to such changes. Dunn (13), however, had shown that no rise in right ventricular pressure occurred in goats after starch embolism, and that there was no conspicuous change in venous or arterial blood pressures. Since these findings are quite consistent with the work of previous investigators, Lichthem (22), Welch (23), Underhill (24), we have not at this time deemed it necessary to inquire into changes in the pressure in the pulmonary circulation for an explanation of tachypnea. Measurement of the pressure in the pulmonary circulation usually requires operative procedures which may in themselves occasion changes in respiratory rates. Haggart and Walker (25) have recently shown that until from 52 to 66 per cent of the pulmonary

circulation is cut off by clamping the pulmonary artery there is no significant variation in the general circulatory condition of the animals (cats) At this point a minute increase in arterial obstruction leads to circulatory collapse with dilatation of the heart and fall in pulmonary and arterial pressure

In 2 dogs receiving intravenous starch injection we observed only a very slight lowering of systemic blood pressure after the onset of tachypnea In 1 of these animals raising the arterial blood pressure by intravenous injection of adrenalin was without effect on tachypnea, showing that this condition is certainly not related to a shock-like fall in systemic blood pressure Figure 7 presents the blood pressure and

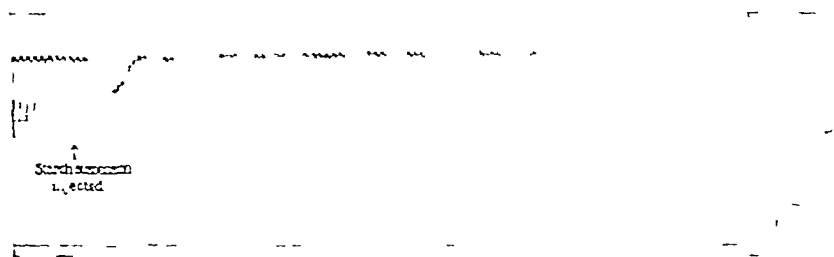


FIG 7 BLOOD PRESSURE AND PNEUMOGRAPHIC TRACING BEFORE AND AFTER INJECTION OF 5 CC 1:4 STARCH SUSPENSION

The mean arterial pressure remains practically unchanged The increase in pulse pressure, decrease in diastolic pressure and marked acceleration of respiratory rate are shown Time marker indicates 1 second intervals

pneumographic tracing of one of these experiments After the injection of 5 cc 1:4 starch suspension there is an immediate drop in systemic pressure which quickly resumes the normal level, to be followed (with onset of tachypnea) by slight lowering of diastolic and increase in pulse pressure, the mean arterial pressure remaining practically unchanged

#### *Reduction of lung volume following starch embolism*

The general picture of congestion suggested a probable change in the elasticity of the lung, such as shows itself in clinical disease by a reduction in vital capacity Since a measurement of vital capacity

requires active cooperation on the part of the subject, it is hardly possible in experimental animals. We were, however, able to measure the volume of air in the lungs at the end of expiration, or the so-called functional residual air (3). This, we know, in man, forms a constant fraction of the total lung volume and varies with it as it does with the vital capacity. In 3 successive lung volume determinations in a normal dog the extreme variations were from 0.55 to 0.52 liter, indicating that the method is reliable when used on animals. A marked reduction of lung volume was observed in a dog which had received an intravenous injection of 9 cc. 1.4 starch suspension. Before injection, when the respiratory rate was 30, the lung volume was 0.52 liter. After starch injection the rate doubled and the lung volume decreased to 0.36 liter, a drop of 30 per cent. One may conclude from this that the hypostasis, edema and swelling of capillaries which has led to atelectasis thereby results in a diminution in air content of the lungs. The probable relation of this to rapid and shallow breathing will be discussed.

#### DISCUSSION

The experiments presented in this paper, we believe, bring out certain facts concerning the causes of rapid and shallow breathing. It has been shown that following the production of multiple emboli of the pulmonary arterioles and capillaries, rapid and shallow breathing ensues. This may be aggravated by anoxemia but does not depend for its existence upon the occurrence of anoxemia. It was thought at the outset that the change in respiratory rate and depth was probably the result of irritative stimuli in the lungs occasioned by the presence of starch granules, and that the effect of vagal freezing which promptly slowed and deepened respiration was to block these afferent stimuli. Two facts which we have observed make us question this interpretation. First, the onset of tachypnea did not occur until a certain volume of starch suspension had been injected, at which time there was gradual acceleration up to a maximum rate. This suggested that the response was related to the mechanical obstruction of the pulmonary circulation, or to the secondary anatomical changes dependent thereon, and that it was not of an irritative reflex nature which might be expected to operate immediately and proportionately to the

dosage The second fact which occasioned doubt as to whether the tachypnea of starch embolism were induced by afferent irritative stimuli was this Other types of tachypnea dependent upon what are probably central stimuli such as anoxic anoxemia and high carbon dioxide concentrations and not dependent upon the presence of foreign bodies in the lungs, are similarly stopped by vagal freezing This, we believe, suggests that an animal whose vagus nerves have been cut or frozen is unable normally to accelerate his respirations, and that he responds chiefly to the fundamental rhythm of the respiratory center which can no longer be notified of changes in the degree of distention and collapse of the lungs, since the Hering-Breuer reflex has been intercepted by vagotomy We have been unable to convince ourselves that the contractions of bronchial musculature which Dunn (13) believed to be of importance are the essential lesions responsible for tachypnea since similar contractions were observed in postmortem examination of the lungs of control dogs

The whole pathological picture in the lungs of these animals is one of vascular congestion and interstitial edema—with localized areas of atelectasis These changes are accompanied by diminution in lung volume and are, we believe, analogous to those seen in disease in human beings in which pulmonary congestion results in loss of elasticity of lung tissue (Lungenstarre) associated with a reduction of the vital capacity

The structural changes resulting in reduced lung volume because of diminished pulmonary elasticity result in mechanical limitation of the depth of the individual breath Thus we see that those conditions in which reduction of lung volume occurs, such as acute and chronic passive congestion of the lungs, lobar pneumonia, pulmonary fibrosis, are the very ones in which the respirations are liable to be shallow and rapid Shallow because of mechanical limitation to distension and collapse, and rapid, we believe, because the normal self-regulating mechanism of Hering and Breuer by which each respiratory phase is terminated and the reverse phase liberated, is sped up by the mechanical limitation of each phase A clearer description of this process is at present hardly possible since we are ignorant of the exact nature of the stimulus to which the Hering-Breuer reflex responds That some such sequence of events can actually occur may be

very simply shown by compressing an anesthetized dog's thorax with the hands or by wrapping an ordinary blood pressure cuff about the thorax and inflating the cuff. Under these conditions the greater the compression, the shallower the breathing, and the shallower the more rapid. This effect is instantaneous and, therefore, almost certainly not the result of chemical changes in the blood or respiratory center. This we conceive to be the mechanism of rapid and shallow breathing as it occurs in multiple experimental embolism of the pulmonary arterioles and capillaries. The condition may be aggravated by anoxemia, but, on the other hand, it may arise independently of anoxemia.

The fact that freezing the vagus nerves will convert such rapid and shallow breathing into slow, deep breathing can be explained on the basis of interference with the Hering-Breuer reflex, with the result that the respiratory center is no longer apprised of the postural state of the lungs. Breathing takes on the fundamental rhythm of the center which tends to be characteristically slow and deep, without the moderating influence of the vagi, and which can, under these conditions, overcome the lung's resistance to distension. It must, of course, be remembered that central stimuli due to the hydrogen ion concentration of the blood and the metabolic needs of the organism will, in part, determine the respiratory rate when depth is limited from whatever cause, no mechanical reflex explanation being sufficient.

It is not improbable that the explanation of the cause of rapid and shallow breathing suggested here obtains likewise in such clinical conditions as acute and chronic passive congestion of the lungs, lobar pneumonia, miliary tuberculosis, pulmonary fibrosis—conditions in which structural changes in the parenchyma lead to loss of elasticity and thus reduction in lung volume, with shallow (and therefore rapid) respirations. To establish this clinical analogy more securely it will be necessary to study the effects of similar experimental structural changes localized in one or more lobes. Such work is in progress, as well as an investigation of the effects of prolonged rapid and shallow breathing with particular reference to the question of fatigue of the respiratory center.

## SUMMARY AND CONCLUSION

1 Multiple emboli of the pulmonary arterioles and capillaries experimentally produced in dogs by the intravenous injection of suspensions of potato starch result in rapid and shallow breathing

2 Such rapid and shallow breathing may be associated with anoxemia of the arterial blood

3 It does, however, not depend upon anoxemia because rapid and shallow breathing persists after anoxemia has been relieved, and because it occurs even when anoxemia has been prevented by previous oxygen inhalation

4 The cause of rapid and shallow breathing following embolism of the pulmonary arterioles and capillaries is therefore not anoxemia

5 Freezing the vagus nerves converts the rapid and shallow breathing of starch embolism into slow, deep breathing

6 This slow, deep breathing does not alleviate the condition of anoxemia which indicates that anoxemia is not caused by rapid and shallow breathing The cause of anoxemia following obstruction to the pulmonary circulation is discussed in Paper II of this series

7 Freezing the vagus nerves of a dog breathing rapidly from oxygen want, due to inhalation of a gas mixture with a low partial pressure of oxygen, similarly results in slow, deep breathing

8 The same effect is produced by freezing the vagi of a dog with tachypnea caused by breathing a gas mixture with a high partial pressure of  $\text{CO}_2$

9 This slowing effect produced by vagal freezing does therefore not necessarily represent the result of blocking afferent irritative peripheral impulses, since in these two instances (7 and 8) the stimulus to rapid breathing was central and chemical

10 It is believed that a dog with vagi frozen is unable to accelerate his respiratory rate

11 Evidence against the starch effect being of an irritative nature is furnished by the fact that a certain critical dose of starch suspension must be injected before the characteristic response of tachypnea occurs

12 The gross and microscopic pathology of "starch" lungs is characterized by evidences of congestion, edema, and atelectasis with multiple emboli occurring in the arterioles and capillaries

13 These changes are not associated with a fall in systemic blood pressure

14 They are associated with a reduction in lung volume (functional residual air) which is believed to be accompanied by a decreased elasticity of the pulmonary parenchyma

15 Such a decrease in elasticity (Lungenstarre) results in shallow tidal air

16 Breathing which is shallow becomes rapid through the mechanism of the Hering-Breuer reflex which depends for its existence upon intact vagus nerves

17 This has been shown by compressing the thorax of an anesthetized dog under which circumstances the greater the pressure the shallower the breathing and the shallower the more rapid

18 An analogy has been suggested between the cause of tachypnea following multiple embolism of pulmonary arterioles and capillaries and the rapid breathing seen in such clinical conditions as acute and chronic congestion of the lungs, lobar pneumonia, miliary tuberculosis, pulmonary fibrosis

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## EXPERIMENTAL STUDIES ON RAPID BREATHING

### II TACHYPNEA, DEPENDENT UPON ANOXEMIA, RESULTING FROM MULTIPLE EMBOLI IN THE LARGER BRANCHES OF THE PULMONARY ARTERY

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#### INTRODUCTION

In Paper I (1) of this series it was shown that multiple emboli of the pulmonary arterioles and capillaries experimentally produced in dogs by the intravenous injection of a suspension of potato starch cells, resulted in rapid and shallow breathing, which occurred independently of anoxemia but was aggravated by it. The primary cause of this type of tachypnea was attributed to anatomical changes in the lung parenchyma in the nature of congestion, edema and atelectasis with reduction of lung volume and diminution in the normal elasticity of lungs. The limitation of respiratory excursion thus produced led to acceleration of rate through the action of the vagus nerves (Herring-Breuer reflex).

An analogy was drawn between this type of rapid and shallow breathing and that seen in such clinical conditions as acute and chronic passive congestion of the lungs, lobar pneumonia, and pulmonary fibrosis.

The object of this present study was to determine whether obstruction to the larger branches of the pulmonary artery would lead to similar effects, or whether they were inherently related to lesions of the arterioles and capillaries. We shall see in this paper that tachypnea does result from obstruction to the larger branches of the pulmonary artery, but that it is different in character and origin from the tachypnea following obstruction to the pulmonary arterioles and capillaries.

## EXPERIMENTAL

In this, as in the previous study, dogs anesthetized with Luminal Sodium were used as experimental animals. To produce obstruction of the larger pulmonary vessels, we resorted to the intravenous injection of seeds of various sizes. The seeds used and their average diameters were as follows

	cm
Poppy	0.1
Rape	0.2
Radish	0.25
Pea	0.5

The method of injecting all seeds except pea seeds was to fill short lengths of glass tubing with a known number of the seeds. The tubing was just wide enough to hold the seeds single file and was of approximately the same bore as the venous cannula. The glass tube and cannula were then filled with 0.85 per cent NaCl solution and the seeds were forced into the vein by flushing out the tube with 10 cc saline from a syringe. To inject the pea seeds, single peas were fixed on a pointed steel wire threaded through a French woven catheter No. 8. The seed was then pushed down the right jugular vein into the right heart. By holding the catheter in place and drawing back the wire the pea was dropped into the cavity of the right heart.

*Embolism of the pulmonary circulation produced in dogs by the intravenous injection of seeds*

Rapid but labored and rather deep breathing associated with progressive cyanosis of the tongue and mucous membranes is the characteristic response of dogs to intravenous injections of seeds. This is true no matter what kind of seed is employed, but with the smaller seeds many more are necessary to bring about this effect than with the larger ones. For example, to raise the respiratory rate from 12 to 29 breaths per minute in one dog, 1000 poppy seeds, averaging 0.1 cm in diameter, had to be injected. Whereas, with pea seeds, which average 0.5 cm in diameter, after 13 had been injected in another animal the rate rose from 12 to 22 breaths per minute. Seeds of intermediate diameter such as rape, averaging 0.2 cm, produced rapid breathing and cyanosis after an intermediate number (122 in one experiment) had been injected. As with starch embolism, so with seeds—a certain dosage had to be injected before any apparent

changes in the animal's condition occurred. For example, in one dog after 50 rape seeds had been injected the respiratory rate and percentage saturation of arterial blood remained unchanged, but with the additional injection of 150 seeds, the respiratory rate trebled and the saturation fell from 89.3 to 72.7 per cent.

In another experiment the injection of 400 poppy seeds had no effect whatever on the respiratory rate and was not accompanied by cyanosis, and marked tachypnea and anoxemia did not occur until nearly 1000 seeds had been injected.

Once tachypnea and anoxemia had arisen there was a tendency for both to progress, with the gradual deterioration of the animal and death from respiratory standstill, the heart continuing to beat for a short period after breathing had ceased. Additional seed injection in a dog already cyanotic and breathing rapidly usually resulted in sudden death.

The fact that a critical number of seeds had to be injected before tachypnea and anoxemia occurred, together with the fact that the greater the number of seeds the severer the effect and, furthermore, that the number of seeds necessary to produce such an effect was in inverse ratio to their diameters, all suggested that we were dealing with a phenomenon directly dependent upon mechanical obstruction to the pulmonary circulation. It remained to show the relationship existing between the two changes that ensued, namely tachypnea and anoxemia, and the causes for their existence. To accomplish this a more precise analysis of the various factors involved was necessary.

(a) *Changes in pulmonary ventilation following seed embolism*  
Unlike starch embolism, seeds produce breathing which is usually labored and deep as well as rapid, and though the rate may rise to 70 or 80 breaths per minute it does not reach the extraordinary degree of rapidity occasionally observed after starch injection. In four experiments the average rate before seed embolism was 14 and after it was 40. This was associated with an average increase in tidal air from 180 to 198 cc. and a resultant change in volume of pulmonary ventilation from 2.62 to 7.97 liters per minute.

(b) *Arterial anoxemia following seed embolism*  
The cyanosis which the dogs exhibited has already been mentioned. That this was dependent upon arterial anoxemia, often of profound degree, was re-

peatedly demonstrated by blood gas analyses. In 12 dogs the average arterial  $O_2$  content before embolism was 16.25 volumes per cent, after embolism it had fallen to 11.59. In these dogs the average capacities remained almost unchanged—the figure before embolism being 18.19 volumes per cent as compared with 18.74 volumes per cent after. This resulted in a decrease in the percentage saturation of arterial blood of nearly 25 per cent, or from an average of 87.56 per cent to one of 62.68 per cent.

(c) *Effect of  $O_2$  inhalation on anoxemia and tachypnea following seed embolism.* It was shown in Paper I (1) of this series that oxygen administration to a dog after embolism of the pulmonary arterioles and capillaries reduced the respiratory rate but little, and that oxygen inhalation before embolism did not prevent the occurrence of tachypnea which, under such circumstances, arose even without the existence of anoxemia. With embolism of the larger branches of the pulmonary artery the effect of oxygen inhalation is quite different from this. To our surprise, in these dogs the respiratory rate was brought down to a normal level on allowing them to breathe oxygen rich mixtures and, furthermore, tachypnea was wholly prevented when oxygen was given prior to the production of emboli. For example, in one dog while breathing room air the respiratory rate was 71 to the minute and the arterial blood was only 53.5 per cent saturated after the intravenous injection of 200 rape seeds. Breathing a 90 per cent  $O_2$  mixture reduced the respiratory rate to 19 and increased the arterial saturation to 97.0 per cent. In another animal the respiratory rate was 13 and the percentage saturation of arterial blood 90.2 before oxygen inhalation and seed injection. The dog was then permitted to breathe 90 per cent  $O_2$ , with the result that his respiratory rate fell to 8, and his arterial blood rose in percentage saturation to 99.2. While in this condition rape seeds were injected intravenously to a total number of 250. The respiratory rate remained at 8 and the saturation fell only to 92.0 per cent which was still in excess of the original. Oxygen inhalation was discontinued, the dog again breathing room air. There resulted a gradual acceleration of respiratory rate and progressive decrease in percentage saturation of arterial blood, these changes occurring simultaneously. In two hours the dog's respirations had reached 57 to the minute, and his arterial blood had decreased 23.7 per cent in saturation with  $O_2$ .

The data of this experiment are presented in table 1

*These facts lead to the obvious conclusion that the cause of tachypnea resulting from seed embolism is anoxemia*

(d) *Effect of vagal freezing upon tachypnea and anoxemia following seed embolism* The effect of vagal freezing was discussed in Paper I

(1) Here we showed that not only the rapid and shallow breathing of starch embolism was converted into slow, deep breathing by freezing the vagi, but that other types of tachypnea resulting from presumably central stimuli, such as low alveolar oxygen or high CO<sub>2</sub> tensions, were similarly checked by this procedure

We suggested that an animal without vagal control was unable to accelerate his respirations. It was reasonable to anticipate that

TABLE 1

*Experiment 36 Intravenous injection of rape seed preceded by oxygen inhalation*

Time	Total number of seeds	Gas inhaled	Respiratory rate per minute	Arterial blood			
				O <sub>2</sub> content	O <sub>2</sub> capacity	Saturation	CO <sub>2</sub> content
				vol per cent	vol per cent	per cent	vol per cent
2 19	0	Room air	13	18.73	20.77	90.2	41.52
3 15	0	90 per cent oxygen	8	18.50	18.66	99.2	48.0
4 55	250	90 per cent oxygen	8	16.16	17.57	92.0	52.6
5 05		Room air	29	13.70	17.36	78.9	48.7
6 08		Room air	42	12.31	17.26	71.3	43.48
6 35		Room air	57	12.49	18.28	68.3	43.2

the rapid breathing following seed embolism, which has been shown to be a sequel of anoxemia, would in like manner be stopped by vagal freezing. This, indeed, is a fact, concerning the truth of which we have repeatedly satisfied ourselves.

*Experiment 11* A female collie weighing 16 kg. was given 1.95 grams Luminal by stomach tube. Three and one half hours later the dog was ready for the experiment, being relaxed and insensitive. The right femoral vein was cannulated for seed injection and the left femoral artery for securing blood samples. The vagus nerves were isolated in the neck.

A pneumographic tracing was obtained, the respiratory rate being 24 per minute. At this time the arterial blood was 89.0 per cent saturated. Radish seeds were then injected intravenously, the respiratory rate accelerating to 76 and the saturation of arterial blood falling to 62.7 per cent. While in this state

the dog's vagi were frozen with the prompt cessation of tachypnea. Respirations now became deep and at the rate of 16 per minute. In spite of the slow, deep breathing the arterial anoxemia progressed, the percentage saturation falling to 34.9 per cent.

Table 2 presents the data of this experiment and figure 1 shows the pneumographic record after anoxemia and tachypnea had progressed to still greater degree. The tracing shows the effect of alternate periods of freezing and thawing the vagus nerves. This experiment shows that the tachypnea resulting from embolic anoxemia can be checked by vagal freezing, and that anoxemia persists in spite of the slow, deep breathing. It suggests, therefore, that the anoxemia is not the result of rapid breathing but of some other cause which will be discussed later.

TABLE 2  
*Experiment 11 Intravenous radish seed injection followed by vagal freezing*

Time	Total number of seeds	Respiratory rate per minute	Arterial blood			
			O <sub>2</sub> content	O <sub>2</sub> capacity	Saturation	CO <sub>2</sub> content
			vol per cent	vol per cent	per cent	vol per cent
1 18	0	24	18.0	20.24	89.0	39.6
2 00	227	76	13.63	21.77	62.7	37.5
2 11		16 (Vagi frozen)	7.85	22.49	34.9	39.9

In another experiment a rate varying from 6 to 9 breaths per minute was maintained for twenty minutes after the intravenous injection of 244 rape seeds in spite of profound oxygen want as indicated by 26.4 per cent oxygen saturation of the arterial blood. The vagi were then thawed and in the succeeding thirty-seven minutes the respiratory rate had accelerated to 76 per minute.

The fact observed thus far in this work and the conclusions to be derived therefrom may be summarized as follows:

- 1 After a certain critical number of seeds are injected intravenously into dogs there results a marked decrease in percentage saturation of arterial blood associated with increase in the respiratory rate.

- 2 The greater the number of seeds, the more severe the reaction, and the smaller the seeds, the greater the number required to bring it about.

3 With small seeds (0.1 cm in diameter) as many as 500 have been injected without producing anoxemia or tachypnea

4 A point is reached, however, when anoxemia occurs and there is an associated acceleration of respirations. These changes tend to be progressive. When anoxemia and tachypnea are established further seed injection frequently results in the death of the animal

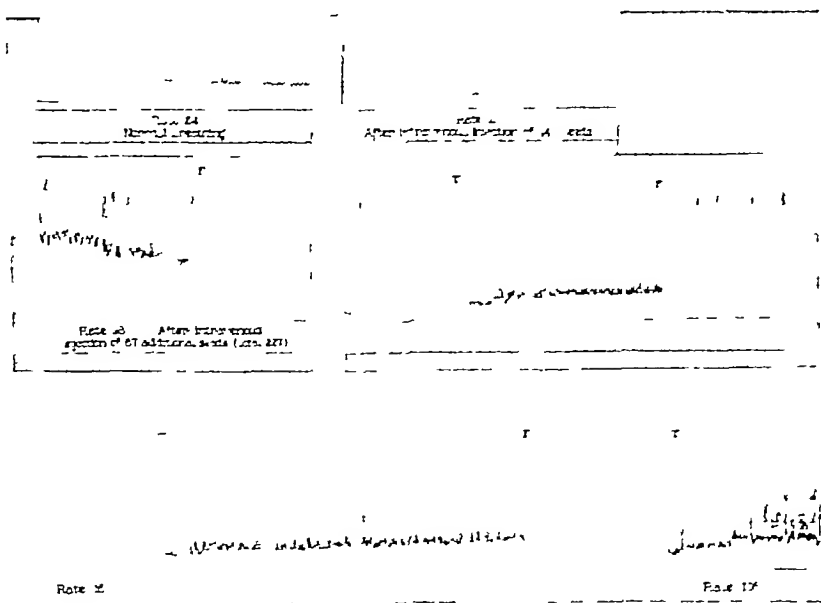


FIG 1 EXPERIMENT 11 PNEUMOGRAPHIC TRACING OF DOG BEFORE AND AFTER SEED EMBOLISM, SHOWING MARKED TACHYPNEA

The letters *F* and *T* indicate alternate periods of freezing and thawing of the vagi. Time marker indicates 5 second intervals. The pneumograph used was made by Joseph Becker, Department of Pharmacology, Columbia University Medical School, N. Y.

These facts all point to the physiological changes being due primarily to obstruction of the pulmonary circulation and coincide with the observations of Haggart and Walker (2) who showed that quantitative occlusion of the pulmonary artery in cats resulted in no significant changes in the circulatory system until from 52 to 66 per cent of the pulmonary circulation is cut off when a sharply defined point



of circulatory collapse occurs involving both pulmonary and systemic pressures

5 Administration of  $O_2$  to a dog thus rendered anoxic and tachypneic, causes a return of the arterial blood to more or less complete saturation with  $O_2$  and reduction in the respiratory rate to a normal level. On subsequent withdrawal of  $O_2$ , the animal again breathing room air, tachypnea and anoxemia recur simultaneously

6 Administration of  $O_2$  prior to and during the production of seed embolism prevents the occurrence of both anoxemia and tachypnea. But these occur as soon as  $O_2$  administration is stopped

These conditions establish clearly the fact that the tachypnea resulting from embolism of the larger branches of the pulmonary artery is wholly the result of anoxemia, thereby differentiating it from the tachypnea resulting from embolism of the pulmonary arterioles and capillaries, which was shown in Paper I (1) to be due to another cause, though, to be sure, often intensified by the existence of anoxemia

7 Freezing the vagus nerves of a dog which is suffering from the rapid breathing and anoxemia of seed embolism immediately slows and deepens the respirations, which again accelerate on thawing the nerves. In spite of slow, deep respirations following vagal freezing, no amelioration of oxygen unsaturation occurs, which progresses as if tachypnea had persisted

The slowing from vagal freezing does not necessarily indicate the blocking of a peripheral irritative stimulus in the lung, as it was shown in Paper I (1) that rapid breathing resulting from central chemical stimuli could be checked similarly by vagal freezing. The general proposition was put forward that an animal without vagal control is unable normally to accelerate his respirations

The fact that slow, deep breathing does not ameliorate the condition of anoxemia indicates that the anoxemia is not the result of tachypnea

It remains for us, therefore, to determine what is the cause of anoxemia following multiple emboli of the larger branches of the pulmonary artery. For this purpose a study of the pathology of the "seed" lungs, both gross and microscopic, has been made, and an attempt, with the aid of numerous injection preparations, to visualize the extent and distribution of the circulatory obstruction

The characteristic feature of these lungs which differentiates them clearly from the "starch" lungs is the absence of generalized congestion, edema, exudate and atelectasis, and the presence of areas of relative ischemia and emphysema. It is probable that in the areas in which circulation persisted, a certain degree of vascular distension and alveolar atelectasis did exist, though this was not definite except in the lungs of dogs surviving several hours after embolism. This latter change corresponds to the congestion observed by Underhill (3) and by Schlaepfer (4) and also by ourselves in the right lungs of animals, of which the left pulmonary artery had been occluded by ligation.

#### PATHOLOGY

Twenty-two animals, injected with different kinds of seeds, were autopsied, the trachea being clamped and the thorax opened immediately after death. The dogs died spontaneously, or were bled to death, or killed by intravenous injection of from 10 to 20 cc. of a saturated magnesium sulphate solution.

#### *Gross pathology*

*Lungs* Disregarding animals which were injected intravenously with dyes before death, notes on the morbid anatomy of the lungs were obtained in 11 animals. Free fluid in the pleural cavity was not present in any instance. In the 3 animals which died within 2 hours after the first seed injection, the lungs showed hypostasis in the dependent (dorsal) parts of the caudal lobes, the remainder of the lobes being salmon pink, well distended, but collapsing readily on removing the tracheal clamp. They were, in fact, normal in appearance. The lung-heart ratio in one of these three dogs was 1.11, i.e., normal (see Paper I). In the remaining 8 dogs in which death occurred from 3½ to 5½ hours after the initial seed injection, hypostasis was more marked than in the previous group. The lungs in the distended state, before the tracheal clamp was removed, showed pale pink areas along the periphery which contrasted with the light reddish areas about the hilum. The lungs collapsed throughout but less so at the periphery than at the hilum. After collapse the surfaces near the hilum were of a dark red color, the periphery remaining pale and emphysematous. The lung-heart ratio in 4 dogs was 1.22, 1.29, 1.34 and 1.55, respectively, while in the animal which lived longest (5½ hours) it was 2.02. This was the only ratio definitely outside the normal range, the increased lung weight being probably due to the hypostatic congestion. In no instance was edema evident.

*Other organs* The right heart was usually dilated. The kidneys, liver and spleen showed no pathological changes referable to the seed injection.

*Examination of injected lungs*

Eight lungs were injected through their pulmonary arteries with barium sulphate gelatine (6 per cent) Examination of the cleared specimens and the x-ray stereoscopic photographs show definitely the areas where the circulation has been blocked (figs 2 and 3)



FIG 2 DOG 8-2 RAPE SEED EMBOLISM PHOTOGRAPH OF SURFACE OF  
INJECTED LUNGS AFTER CLEARING IN OIL OF WINTERGREEN

Note the numerous blocked areas where the barium sulphate gelatine has not penetrated

The pulmonary arterial bed of one other lung was washed out with saline and the lung dehydrated and cleared in oil of wintergreen. In this transparent specimen the seeds may be clearly seen. They are widely distributed but tend to line up in rows, one behind the other, in the main peripheral vessels. The distribution of the seeds may, however, best be seen in 4 specimens in which the pulmonary artery was injected with celloidin and the lung tissue digested away.



FIG 3 DOG 9-2 RAPE SEED EMBOLISM X-RAY PHOTOGRAPH OF LUNGS  
INJECTED WITH BARIUM SULPHATE GELATINE

Note the numerous un.injected areas. These are mostly at the periphery as the seeds tend to follow and lodge in the main arterial trunks, the diameters of which gradually decrease in size.

with muriatic acid (Hinman, Morison and Lee-Brown (5)) The seeds adhered to the celloidin and remained undigested Their exact situation could, therefore, be observed

To obviate the artificial conditions of postmortem injections, 3 dogs were infused intravenously during life with from 65 to 100 cc dialyzed India ink



FIG 4 DOG 55-2 PEA SEED EMBOLISM PHOTOGRAPH OF SURFACE OF LUNG AFTER ANTEMORTEM INTRAVENOUS INJECTION OF INDIA INK

Note the comparatively small area around the hilum stained black where the pulmonary circulation was not obstructed

(Krogh (6)) Examination of their lungs within 5 minutes showed that large parts of the peripheral portions were unstained, in contrast with the remainder of the lungs which were black This corresponded to the picture in postmortem injections A photograph of such an India ink preparation is reproduced in figure 4

*Microscopical pathology*

*Lungs* Histological examination was made in 7 cases. In no instance was edema or exudate noted. The branches of the pulmonary artery were frequently completely blocked by the presence of seeds and enveloping clots, the calibre of the artery obstructed being dependent on the size and number of seeds injected. In the 4 instances in which the experiment was concluded within 2 hours of seed injection, it was difficult to distinguish the blocked from the unobstructed vascular areas. It appeared as if the obstructed areas were relatively ischemic and emphysematous and, by contrast, the vessels of the unobstructed areas appeared congested and the alveolar walls partially collapsed. This contrast was more pronounced in the 3 dogs which survived longer than 2 hours.

The hypostatic areas were not included in the above description. The muscular contractions noted in the bronchiolar walls of normal and "starch" lungs were also observed here.

## EMBOLIC ANOXEMIA

In their monograph Lundsgaard and Van Slyke (7) have made a detailed theoretical study of the factors which contribute to the occurrence of cyanosis. Whereas the appearance of clinical cyanosis should be differentiated from anoxemia as measured by the percentage saturation of the arterial blood, it is nevertheless true that many of the same factors are involved in the production of both. An enumeration and analysis of these will help us to understand the causes which give rise to embolic anoxemia.

Of the factors which may be responsible for arterial anoxemia the more important are these:

- 1 Low alveolar oxygen tension due to (a) diminished atmospheric oxygen pressure, (b) inefficient ventilation

- 2 Retardation of diffusion of oxygen from alveoli into blood due to presence of edema or exudate

- 3 Shunt or passage of a fraction of blood through unaerated channels from the venous to the arterial system. This may be (a) complete, in which no aeration of shunted fraction occurs, or (b) partial, in which partial aeration of shunted fraction occurs.

- 4 Increased reduction of oxygen during flow through the tissue capillaries due to (a) greatly increased rate of oxygen consumption by the tissues, (b) decreased rate of flow through the tissue capillaries.

5 A change in the total content of haemoglobin, which if increased would tend to decrease the percentage saturation of the blood passing through the lungs

6 A change in the quantitative relation of blood flow to vascular diffusion area in the lungs

An analysis of the rôle played by these various factors in the production of *embolic anoxemia* follows

### 1 *Low alveolar oxygen tensions*

(a) *Diminished atmospheric oxygen pressure* There can be no question of this entering as a cause of the type of anoxemia here described as in all the experiments the dogs breathed either room air or gas mixtures with a higher partial pressure of oxygen than exists in room air

(b) *Inefficient ventilation* On page 157 it was shown that the response of breathing to seed embolism is characterized by increased depth as well as accelerated rate. Under such circumstances there should be no diminution of the effective ventilation. From this alone one might conclude that anoxemia was not the result of inefficient ventilation. Further evidence for this is furnished by the vagal freezing procedure in which a 37 per cent increase in the depth of tidal air did not increase the percentage saturation of arterial blood.

In addition to these observations the following experiment was performed to eliminate inefficient ventilation as a cause for the anoxemia observed.

*Experiment 68* A dog was rendered anoxemic by the intravenous injection of 169 rape seeds. His respiratory rate, which had been 9, rose to 37 and his arterial blood, which had contained 15.54 vol per cent of  $O_2$ , now contained 10.27 vol per cent. The resulting decrease in percentage saturation was from 96.00 to 61.01 per cent, the capacity having increased by only 0.60 vol per cent. While in this condition, artificial ventilation by the intratracheal insufflation of air at the rate of 27 liters a minute was maintained for 15 minutes, when another sample of arterial blood was drawn. This showed no amelioration of the anoxemia. The  $O_2$  content was 9.99 vol per cent and saturation 59.10 per cent.

This experiment together with the foregoing observations we believe definitely rules inefficient ventilation as the cause of embolic anoxemia.

## 2 *Retardation of diffusion of oxygen from alveoli into blood due to presence of edema or exudate*

The solution of this phase of the problem depended largely upon the gross and microscopic pathology of the embolized lungs. In postmortem examination of twenty-two dogs rendered anoxic by intravenous injection of seeds, none was found in which there was free fluid in the pleural cavities or gross evidence of interstitial edema. The cut surfaces of the lungs were not more moist than normal, nor did fluid exude from the trachea or bronchi. Furthermore, the weight ratios of lungs to heart were normal in those dogs in which this observation was made at autopsy.

In the microscopical examination of the lungs of seven dogs which had been rendered anoxic by intravenous seed injection, no evidence was found of intra-alveolar exudate or of change in the appearance of the alveolar wall other than a certain degree of emphysema.

An actual measurement of the diffusion constant as has been made in the human subject by Marie Krogh (8) was not possible, as this method requires precise coöperation on the part of the subject and is, therefore, not applicable to experimental animals. Krogh has shown that the rate of diffusion of a gas through the alveolar epithelium depends upon its partial pressure and is directly proportional to the surface of epithelial tissue and inversely proportional to its thickness or to the thickness of the alveolar wall. The surface area of epithelial tissue Krogh expressed as the two-thirds power of the mean alveolar lung volume (usually called the mid capacity). She showed that increase of the alveolar lung volume above the mean increased the diffusion constant, but decrease below the mean did not alter it because, she argued, that such a decrease, in the normal lung, was due to folding and wrinkling of the alveolar walls which would not reduce their surface area. This argument was substantiated by experimental observations.

Though the actual diffusion constant could not be measured in these experiments, it was thought desirable to measure the lung volume. It was believed that lung volume estimation might furnish at least indirect evidence of any changes in the area of alveolar epithelium available as a diffusion membrane.

In each of three experiments an *increase in lung volume (functional residual air)* (9) accompanied the anoxemia following seed embolism. These changes in lung volume, respiratory rate and percentage



saturation of arterial blood are presented in table 3. This augmentation of lung volume is consistent with the gross and microscopic evidence of emphysema. It should be compared with the successively constant lung volume determinations made on a control dog, and the decreasing lung volumes resulting from congestion, edema and atelectasis following starch injection (see Paper I of this series).

In the absence of gross or microscopic evidence of edema or of exudative changes, and in the presence of an increase in lung volume suggesting no diminution in the surface area of alveolar epithelium available for the diffusion of gases, it seems reasonable to

TABLE 3  
*The effect of seed embolism on lung volume, respiratory rate and arterial O<sub>2</sub>*

Experiment number	Time	Total seeds injected	Functional residual air	Respiratory rate per minute	Arterial blood		
					O content	O <sub>2</sub> capacity	Saturation
			<i>liters</i>		<i>vol per cent</i>	<i>vol per cent</i>	<i>per cent</i>
34	1 42	0	0 48	28	11 17	15 33	72 9
	2 39	155	0 51	33	9 18	16 15	56 8
35	12 44	0	0 56	8	15 15	17 02	89 0
	1 41	50	0 61	9	15 00	16 80	89 3
	3 18	200	0 70	27	12 82	17 64	72 7
	4 07		0 87	71	10 39	19 43	53 5
36	2 30	0	0 73	13	18 73	20 77	90 2
	6 40	250	0 78	57	12 49	18 28	68 3

conclude that anoxemia of embolic origin is *not the result of retardation of diffusion of oxygen from the alveoli into the blood due to changes in permeability of the alveolar walls*.

### 3 Shunt of blood through unaerated channels

(a) *Complete shunt or passage of a fraction of blood through completely unaerated channels from the venous to the arterial system*. As a possible cause of embolic anoxemia this may be quickly disposed of by the experiments with inhalation of high concentration of O<sub>2</sub>. On page 158 it was shown that breathing 90 per cent O<sub>2</sub> raised the saturation of the arterial blood from 53.5 to 97.0 per cent. This increase in

saturation was repeatedly observed. Were blood being shunted from the venous to the arterial system through completely unaerated channels, raising the alveolar  $O_2$  tension alone could not thus cause the  $O_2$  unsaturation to disappear.

(b) *Partial shunt or passage of a fraction of blood through partially unaerated channels from the venous to the arterial system.* That this is not responsible for the occurrence of arterial anoxemia after pulmonary embolism is difficult to establish definitely. There is, however, strong presumptive evidence against it.

1 There is no morphological appearance of partial obliteration of alveolar spaces, nor is there any evidence of bronchiolar or atrial spasm.

2 There is no decrease but, in fact, an increase of lung volume after seed embolism (see table 3).

3 There was no apparent delay after embolism in obtaining equilibrium between lung air and the hydrogen-oxygen mixture contained in the spirometer used for lung volume determinations. This suggests that the alveolar air was accessible to atmospheric air and that partially unaerated portions of the lung did not exist.

4 The fact that artificial respiration by intratracheal insufflation did not relieve the arterial  $O_2$  unsaturation is strong evidence against the existence of unaerated areas as these would in all probability have been ventilated by the method of intratracheal insufflation.

#### *4 Increased reduction of oxygen during flow through the tissue capillaries*

(a) *Increased rate of oxygen consumption by the tissues.* By actual measurement of the metabolic rate existing before and after seed embolism it was shown that there was no consistent change in oxygen consumption by the tissues. In one dog 74.55 cc  $O_2$  were consumed per minute when the arterial blood was 90.8 per cent saturated, and 74.40 cc  $O_2$  were consumed when the saturation had fallen to 73.3 per cent. In another dog, a drop in percentage saturation from 82.2 to 42.5 per cent was accompanied by a decrease in  $O_2$  consumption from 80.06 to 72.77 cc per minute, and in a third dog a decrease in percentage saturation from 97.7 to 59.2 was associated with a rise

in  $O_2$  consumption from 101.53 to 134.33 cc per minute. For data of these three experiments table 4 should be consulted. They indicate that the type of anoxemia with which we are dealing is not the result of increased tissue metabolism.

(b) *Decreased rate of flow through the tissue capillaries* That this in itself can be a cause of arterial anoxemia is improbable without the concomittant changes in the pulmonary circulation which will be discussed under heading 6. A slowing of rate of flow through the tissue capillaries might result in anoxemia of the capillary blood, but it is difficult to see how it could result in arterial anoxemia. A slow

TABLE 4

*Measurement of  $O_2$  consumption before and after anoxemia following seed embolism*

Experiment number	Rectal temperature	Total number of seeds injected	Respiratory rate per minute	$O_2$ consumption per minute	Arterial blood			
					$O_2$ content	$O_2$ capacity	Saturation	$CO_2$ content
					vol per cent	vol per cent	per cent	vol per cent
49	38.0	0	12	70.85	18.31	18.10	100	49.01
	38.2	205	24	74.55	16.89	18.61	90.8	49.09
	38.0	255	34	74.40	13.68	18.67	73.3	46.1
50	38.5	0	12	80.06	14.33	17.44	82.2	43.2
	36.8	235	46	72.34	11.04	16.56	66.7	44.62
	35.5		42	72.71	7.44	17.51	42.5	46.28
51	39.2	0	22	101.53	17.24	17.65	97.7	42.23
	38.0	300	48	125.95	13.19	18.40	71.7	
	38.5		46	134.33	12.55	21.20	59.2	39.22

rate of flow through the systemic capillaries, if accompanied by a slow rate of flow through the pulmonary capillaries, should enable the reduced hemoglobin to be reoxygenated in the lungs.

That there is no such actual decrease in rate of flow through the tissue capillaries is suggested by the following facts:

1. No marked decrease in output of the heart per beat or per minute was observed after seed embolism (see below under 6).

2. No marked fall in systemic blood pressure was observed after the production of anoxemia due to seed embolism as is shown in figure 5, the mean arterial pressure before embolism being 81 mm Hg and after 79 mm Hg.

5 *A change in the total content of hemoglobin which if increased would tend to decrease the percentage saturation of the blood passing through the lungs*

That such an increase is not the cause of embolic anoxemia is shown by the following facts

In 12 dogs in which embolic anoxemia was produced the average changes in arterial blood were as follows. Before embolism the arterial  $O_2$  content was 12.25 vol per cent as compared with 11.59 vol per cent after. The  $O_2$  capacity, on the other hand, had increased very slightly, being 18.19 vol per cent before as compared with 18.74

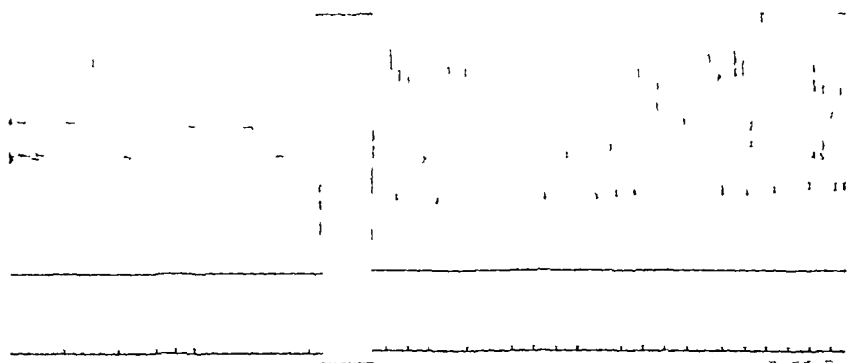


FIG 5 BLOOD PRESSURE AND PNEUMOGRAPHIC TRACINGS BEFORE AND AFTER TACHYPNEA FOLLOWING SEED EMBOLISM

Time marker indicates 5 second intervals

vol per cent after embolism. This represents an increase in hemoglobin of only 0.3 per cent whereas the decrease in percentage saturation was from 87.56 to 62.68 or approximately 25 per cent.

A control experiment to check this point was done by bleeding a 9 kg dog 150 cc of arterial blood at the height of embolic anoxemia when his blood was 59.10 per cent saturated. No increase in percentage saturation resulted but, in fact, a decrease to 56.20 per cent.

At this point in the paper a résumé of the foregoing argument may appear desirable. It has been shown that multiple experimental emboli of the larger branches of the pulmonary artery in dogs gives rise to *anoxemia* and thus to *tachypnea*. An effort to explain the cause of this anoxemia has shown that it is not related to

- 1 Low alveolar oxygen tension
- 2 Retardation of diffusion of oxygen from alveoli into blood due to edema or exudate
- 3 Shunt or passage of fraction of blood through either completely or partially unacrated channels from the venous to the arterial system
- 4 Increased reduction of oxygen during flow through the tissue capillaries
- 5 Increase in the total content of hemoglobin

It is believed that embolic anoxemia results from the sixth cause enumerated above, namely, *a change in the quantitative relation of blood flow to the vascular diffusion area in the lungs*. The following experimental data and discussion will essay to establish this point.

It should be stated at the outset that anything approaching a quantitative estimation of the size of the capillary diffusion area of the lungs has not been possible and we have had to depend upon the morphological appearance of injected specimens to give us a conception of the extent of obstruction to the pulmonary circulation caused by seed embolism. Photographs of such specimens are shown in figures 2, 3 and 4. There can be no doubt from these specimens that the emboli have set up an effective blockade which prevents the passage of blood and thus much diminishes the vascular diffusion area. In the specimen shown in figure 4 it may be estimated that approximately two thirds of the vascular bed of the lungs has been obstructed.

The relation between blood flow and the area of the pulmonary vascular bed has been expressed by Stewart (10) in the following formula

$$T \approx \frac{Q}{rQ'}$$

where  $Q$  = capacity of the pulmonary circulation,

$Q'$  = average output of the right ventricle,

$r$  = the number of beats of the heart per second, and

$T$  = the pulmonary circulation time

It appears from this that a reduction in  $Q$  such as we are here dealing with,  $r$  and  $Q'$  remaining constant, would result in a diminution of  $T$  or, in other words, in a more rapid flow of blood through

the pulmonary circulation. The possibility should be borne in mind that the rate of flow may be so fast that the blood cannot take up its usual load of oxygen. Indeed from the recent considerations of L J Henderson and his co-workers (11) on the time of the diffusion process in the lung this interpretation may be the correct one.

An actual estimation of the changes in  $Q'$  or at least of the changes in cardiac output per beat, was made in three experiments before and after seed embolism by the method of Barcroft, Boycott, Dunn, and Peters (12). In these experiments temporary changes in volume of cardiac output and in the volume of blood flowing through the lungs occurred after seed embolism which did not persist in spite of the persistence of anoxemia. It is safe to say, therefore, that such transitory changes were not responsible for the occurrence of anoxemia. In the first experiment of the three, the cardiac output per beat decreased from 11.3 to 9.9 cc before and after seed injection, respectively. This amounted to a reduction in blood flow through the lungs per minute from 1.61 to 1.35 liters. In the second experiment the changes were of a similar order of magnitude. Before seed embolism the cardiac output per beat was 19.1 cc after 17.0 cc and the blood flow per minute through the lungs was 2.73 liters as compared with 2.18 liters. In the third experiment 12.5 cc of blood were being delivered from the heart before embolism as compared with 11.6 cc after, and 2.51 liters of blood were flowing through the lungs per minute before embolism as compared with 2.32 liters after. The data of these three experiments are presented in table 5. In each case a slight diminution in cardiac output per beat and in minute volume blood flow through the lungs resulted. If we pool the data of these three experiments we obtain the figures in table 6.

Let us assume that in these three experiments half of the total pulmonary vascular bed has been obstructed by seeds and that 100 arbitrarily represents the capacity of the pulmonary circulation,  $Q$ , before embolism. We have then

*Before embolism Circulation time*

$$T \propto \frac{100}{37.18} = 2.69$$

*After embolism Circulation time*

$$T \propto \frac{50}{32.00} = 1.56$$

Experiment num ber	Time	Total number of seeds injected	O <sub>2</sub> content of blood		O <sub>2</sub> capacity of blood		Saturation of blood		CO <sub>2</sub> content of blood		Respiratory rate per minute	Heart rate per minute	Tidal air cc	Minute volume liters	O <sub>2</sub>	A - V <sup>*</sup>	F <sup>*</sup>	Cardiac output per beat	CO <sub>2</sub> produced per minute
			Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous									
49	11 27	0	18 31	13 93	18 10	18 09	100 00	77 00	49 01	49 01	12	142	170 0	2 05	70 85	0 44	1,610	11 3	60 24
	2 44	205	16 89	10 45	18 61	18 76	90 80	55 70	49 09	48 30	24	144	189 0	4 54	74 55	0 64	1,164	8 1	75 38
	4 07	255	13 68	9 18	18 67	20 04	73 30	45 80	46 10	50 70	34	136	186 0	6 32	74 40	0 55	1,352	9 9	67 36
50	1 24- 1 34	0	14 3	11 40	17 44	14 66(?)	82 20	77 80(?)	43 20	46 10	12	143	187 5	2 25	80 06	0 29	2,730	19 1	70 47
	3 14- 3 24	235	11 04	5 41	16 56	16 11	66 70	33 50	44 60	44 78	46	139	172 4	7 93	72 34	0 56	1,284	9 2	3 51
	3 55- 4 05		7 44	4 10	17 51	18 34	42 50	22 34	46 28	48 52	42	128	164 0	6 89	72 71	0 03	2,177	17 0	3 67
51	2 03- 2 13	0	17 24	13 02	17 65	16 78	97 70	77 60	42 23	42 23	22	192	179 0	3 94	101 53	0 42	2,405	12 52	77 05
	4 49- 4 54	300	13 19	8 67	18 40	19 40	71 70	44 69		43 63	48	186	217 0	10 42	125 95	0 45	2,786	14 96	5 60
	5 37- 5 44		12 55	6 77	21 20	21 39	59 20	31 62	39 22	43 05	46	200	229 7	10 57	134 33	0 57	2,324	11 61	5 68

\* In this table  $F = \frac{O}{A - V}$  where  $A - V$  = the difference in content of O<sub>2</sub> between arterial and venous blood in cubic centimeter per 1 cc blood  $O$  = the total O<sub>2</sub> in cubic centimeter used per minute, and  $F$  = the cubic centimeter of blood flowing through the lungs per minute

This represents a 42 per cent decrease in time (increased rapidity) of blood flow through the pulmonary vessels. Such a change alone, according to Henderson's approximations, would be responsible for, roughly, a 10 per cent decrease in volume per cent  $O_2$  content of arterial blood. There is, however, another factor involved besides increased rapidity of blood flow. For the argument we have advanced assumes that no compensatory dilatation of unobstructed vessels has occurred. But our whole knowledge of the behavior of capillaries leads us to believe that this is not the case.

The postmortem findings of Underhill (3) and of Schlaepfer (4) give definite evidence for the occurrence of a compensatory dilatation of vessels in the intact lung after the artery to the other lung has been ligated. Since under these conditions there is usually a 40 per cent rise in pulmonary blood pressure with no effect on carotid pressure,

TABLE 6

	Heart rate per second	Cardiac output per beat	$r Q$ or cardiac output per second
		cc.	cc.
Before embolism	2.6	14.3	37.18
After embolism	2.5	12.8	32.00

pulse rate, output of the heart or state of its dilatation, Underhill concludes that the healthy heart can accommodate itself by sending the same volume of blood through one lung in a given time as it previously sent through both. In our own experiments, which were of relatively short duration, difficulty in differentiating histologically the obstructed from the unobstructed areas in uninjected specimens made the actual demonstration of such dilated vessels somewhat uncertain. It is reasonable however, to assume the existence of a compensatory dilatation in the unobstructed vessels. Though in a lung, such as that shown in figure 4, it is scarcely conceivable that dilatation could be sufficient to restore the vascular bed to its original capacity. In such dilated capillaries crowded with corpuscles the inward diffusion of  $O_2$  should be impaired and this should form a contributing cause to the type of anoxemia with which we are dealing here. Both these causes, which result in anoxemia, i.e., increased



rate of flow and increased blood bulk in the capillaries, could be corrected by raising the alveolar oxygen tension

It should be stated here that Underhill (3) observed the occurrence of anoxemia in the blood of cats after ligating the artery to the left lung. Artificial ventilation did not relieve this anoxemia when the chest was closed. Underhill made no attempt to explain the cause of the anoxemia, but stated, as we have already mentioned, that under these conditions the right lung contained more blood than normal. With the chest open he found it was possible for the blood to be 90 to 95 per cent saturated provided sufficient ventilation were being given

#### CO<sub>2</sub> CONTENT OF ARTERIAL BLOOD FOLLOWING SEED EMBOLISM

In spite of the impaired inward diffusion of oxygen due to the diminution of the vascular area, the carbon dioxide content of the arterial blood in these dogs remained remarkably constant. In 10 dogs in which seed embolism and the resulting anoxemia was produced the average content in CO<sub>2</sub> of the arterial blood before embolism was 44.46 volumes per cent as compared with 43.26 volumes per cent after embolism. The fact that there was no "piling up" of CO<sub>2</sub> in the blood can best be explained by (a) the existence of hyperventilation, (b) the greater diffusibility of CO<sub>2</sub> than O<sub>2</sub> due to its greater solubility coefficient. A normal or low CO<sub>2</sub> content of arterial blood in the presence of anoxemia is commonly seen in patients with lobar pneumonia.

#### DISCUSSION

As is suggested by the title of this paper and Paper I two distinct causes of rapid breathing have been encountered (a) resulting from embolism of pulmonary arterioles and capillaries, (b) resulting from embolism of the larger branches of the pulmonary artery. The first is dependent on reflex changes arising from structural modifications in the pulmonary parenchyma. The nature of these is a generalized congestion and edema which produces a reduction in lung volume and an impairment of the normal elasticity of the lungs. This tends to limit the extent of the respiratory excursion and, as we have explained, thereby to quicken respirations. The second cause of rapid breathing, with which we have dealt in this paper, is anoxemia which results from a reduction in the vascular diffusion area. This second

type may be a complicating factor of the first. To both there are clinical analogies. Acute and chronic congestion of the lungs, lobar pneumonia, pulmonary fibrosis, are clinical conditions characterized by rapid respirations resulting, we believe, in part at least, from reflex effects due to the mechanical limitations to inflation and deflation. Each of these conditions may be associated with anoxemia which, if present, will tend to a further acceleration of respiratory rate.

The causes of cyanosis and anoxemia as clinically seen have been fully discussed by Lundsgaard and Van Slyke (9). That obstruction to the pulmonary circulation *per se* may constitute such a cause was not emphasized by them, and yet in such conditions as post-operative pulmonary embolism where there is often intense cyanosis accompanied by rapid breathing, which can and should be relieved by continuous oxygen therapy, there can be little doubt that we are dealing with a phenomenon analagous to the one experimentally produced in this work. To what extent the obliteration of the branches of the pulmonary artery which exists in pneumonia is responsible for the occurrence of anoxemia and cyanosis, we are not prepared to say, but it is probable that it plays a rôle.

#### SUMMARY AND CONCLUSIONS

1 Multiple emboli of the larger branches of the pulmonary artery experimentally produced in dogs by the intravenous injection of seeds of various sizes results in tachypnea and anoxemia.

2 The tachypnea is due to the anoxemia and can be stopped or prevented by oxygen inhalation.

3 The anoxemia has been attributed to a change in the quantitative relation of blood flow to the vascular diffusion area in the lungs. The nature of this changed relationship is twofold: (a) an increased rate of flow through the capillaries, the flow being so rapid that the blood cannot assume its normal load of oxygen, (b) a compensatory dilation in the capillaries which are crowded with corpuscles in columns so thick as to interfere with the normal inward diffusion of oxygen. Each of these defects in  $O_2$  diffusion can be remedied by raising the alveolar  $O_2$  tension.

4 Changes of this sort occur in such clinical conditions as lobar pneumoma and pulmonary embolism where cyanosis and rapid breathing are commonly encountered and where oxygen therapy is indicated

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# TRANSIENT AURICULAR FIBRILLATION FOLLOWING DIGITALIS THERAPY, WITH OBSERVATIONS UPON THE REACTION TO ATROPINE

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## INTRODUCTION

There is some evidence that the onset of auricular fibrillation in man during the course of digitalis therapy may be dependent upon the administration of this drug. There are a few cases in the literature in which this relationship is suggested. Mackenzie (1910-1911) was the first to describe a case in man in which auricular fibrillation occurred during the course of digitalis therapy. In his patient, auricular fibrillation appeared at the height of the digitalis effect and disappeared three days after the drug was discontinued. Danielopolu (1916) reported three cases in which auricular fibrillation appeared following toxic doses of digitalis, but no statement was made as to the disappearance of auricular fibrillation after the withdrawal of the drug, so that the dependence of the irregularity of rhythm upon the use of digitalis is not clear. Reid (1923) reported two cases, in only one, however, did the normal rhythm return after the omission of the drug. Similar cases have been described by Cushny (1911, a, 1911, b), Robinson (1914), Krumbhaar (1916) and others, but with the possible exception of one case mentioned by Cushny, the data presented are either inconclusive or are too meagre for an accurate estimation of the influence of digitalis in producing auricular fibrillation. Experimentally, this phenomenon has been noted by Cushny (1911, b).

## METHOD OF STUDY

During the past year, several cases of auricular fibrillation have followed the giving of digitalis in this hospital. In order to investigate certain problems connected with the use of digitalis, a more or less

routine plan of procedure was adopted. For several days after admission the patient was treated by the usual means such as rest in bed, restriction of diet and fluid intake except that digitalis was not administered. He was then given the drug in relatively large amounts so that he was brought rapidly under the influence of digitalis. The drug was continued until a definite effect was obtained such as vomiting, slowing of heart rate, clinical improvement, electrocardiographic changes. In a few instances, it became necessary therefore to administer more than the average therapeutic dose of digitalis, but an attempt was made by careful clinical observation and by frequent electrocardiographic records to avoid any serious effects. For various reasons, the preliminary digitalis-free period was omitted in some patients, in a few instances, the condition of the patient was so serious that it was thought necessary to give the drug at once. The preparation of digitalis used was a powdered leaf of high potency, having a cat unit of 0.065. Clinically, it was found in the study of a fairly large number of patients that within moderate limits, a definite digitalis effect was seen after the use of 0.015 gram (or slightly less) per pound of body weight. In calculating the relation of dosage to weight, figures for the latter have been taken after the edema disappeared, or when it persisted, an approximate deduction has been made. An average daily excretion of 0.15 gram was assumed (Pardee, 1919). In all but two of the patients forming the basis of this paper, the reaction to atropine was studied following the onset of auricular fibrillation. Atropine was given either hypodermatically, intramuscularly or intravenously, in varying doses, but never less than 2 mgm. In all the observations upon the effect of atropine electrocardiographic records were taken at frequent intervals over a period of 60 to 90 minutes.

Only those cases observed personally by the writer have been included in this report, and these happened all to be on the wards for colored patients.

#### CLINICAL OBSERVATIONS

It may be stated at once that all patients described below were subjects of an advanced degree of myocardial failure. Digitalis was discontinued shortly after the discovery of auricular fibrillation, and the irregularity disappeared spontaneously in every instance after the drug was withdrawn.

Case 1 was a man suffering from cardiovascular disease and arterial hypertension. On giving digitalis, he developed 2:1 auriculo-ventricular block which was followed shortly by the appearance of auricular fibrillation. This persisted for six days and then disappeared (table 1). During the interval in which the auricles were fibrillating, the reaction to atropine was observed on two occasions. In the first test, the ventricular rate rose from 53 to 70 following the intravenous injection of atropine 3 mgm., given in divided doses of 2 and 1 mgm. Practically identical results were obtained in the second test, in which a single dose of atropine 3 mgm. was given (fig. 1). During the remainder of his stay in the hospital the rhythm

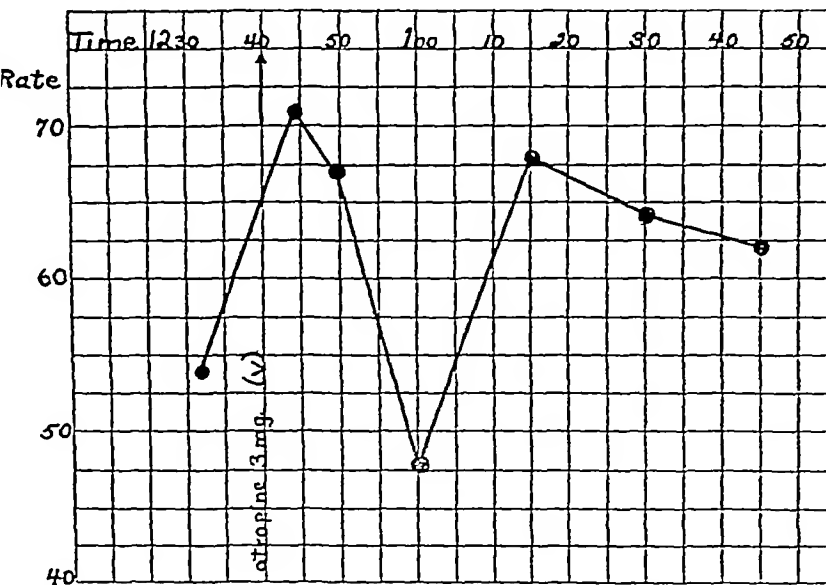


FIG 1 CASE 1 SECOND ATROPINE TEST

This chart illustrates the effect of atropine 3 mgm given intravenously on the ventricular rate

remained normal. On a second admission, three months later, the heart rhythm was found to be normal. Again, after giving digitalis, auricular fibrillation appeared. A third injection of atropine 3 mgm given intravenously caused the rate to rise from 60 to 84. The irregularity persisted for six days, after which the rhythm remained normal.

Case 2 was a woman with cardiovascular disease and arterial hypertension. Following the first period of digitalis administration, there was marked improvement. This was temporary, however, and she became progressively worse until death occurred. At the end of the second period of digitalis administration, 3 2

auriculo-ventricular block appeared, and on the following day, 24 hours after the last dose of digitalis, the heart block was complete. It disappeared on the following day. During the third period of treatment with digitalis, when the patient's condition had become serious and the degree of cardiac failure was marked, auricular fibrillation developed and persisted for 26 days, disappearing six days after strophanthin was discontinued. The effect of the administration of 4 mgm of atropine during the period of fibrillation was to increase the ventricular rate from 68 to 102 (fig 2)

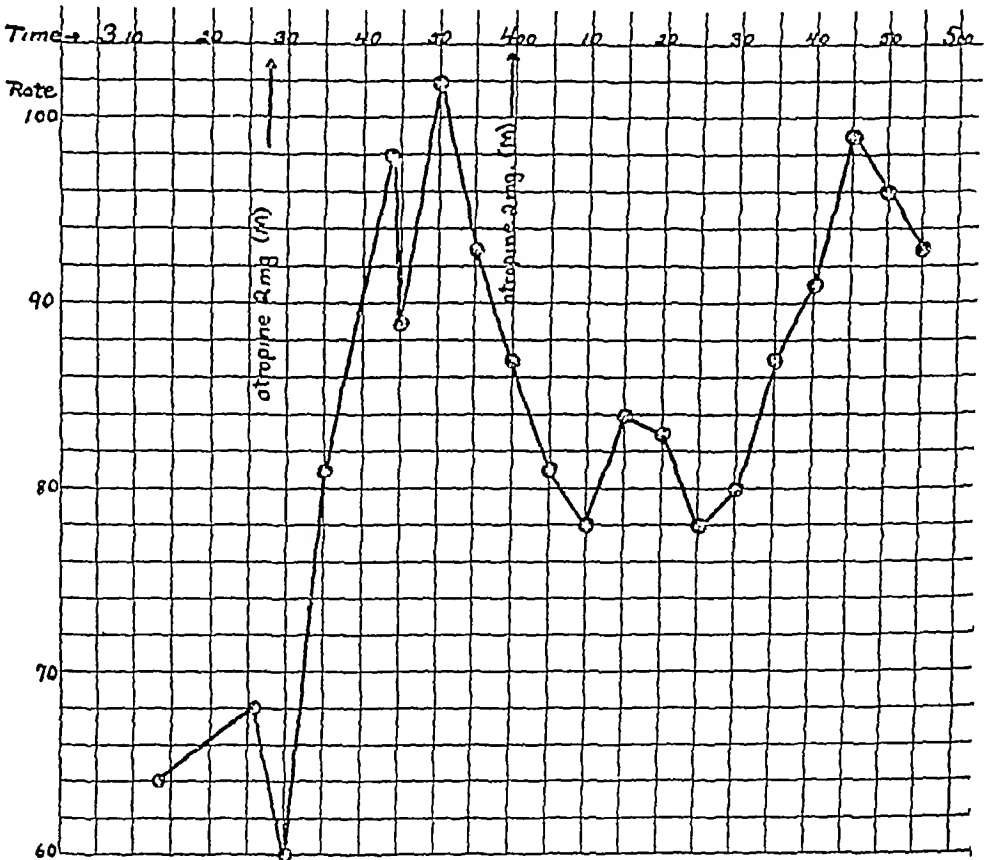


FIG 2 CASE 2

This chart shows the effect of the intramuscular injection of atropine 2 mgm, followed by a second similar dose, on the ventricular rate

At the end of the last course of strophanthin administration, on the day of her death, the normal rhythm changed to auricular flutter. This took place after comparatively small doses of strophanthin, but when the patient's heart failure was extreme. The rate of the fluttering auricle was unusually low, 165. This occurred in the presence of a terminal infection, the rôle of which it is impossible to estimate in the production of this abnormal rhythm.

Cases 3, 4, 5 and 6 were men with syphilitic heart disease. In all, auricular fibrillation appeared very shortly after the administration of effective doses of digitalis. During the period of fibrillation, the effect of atropine was observed in

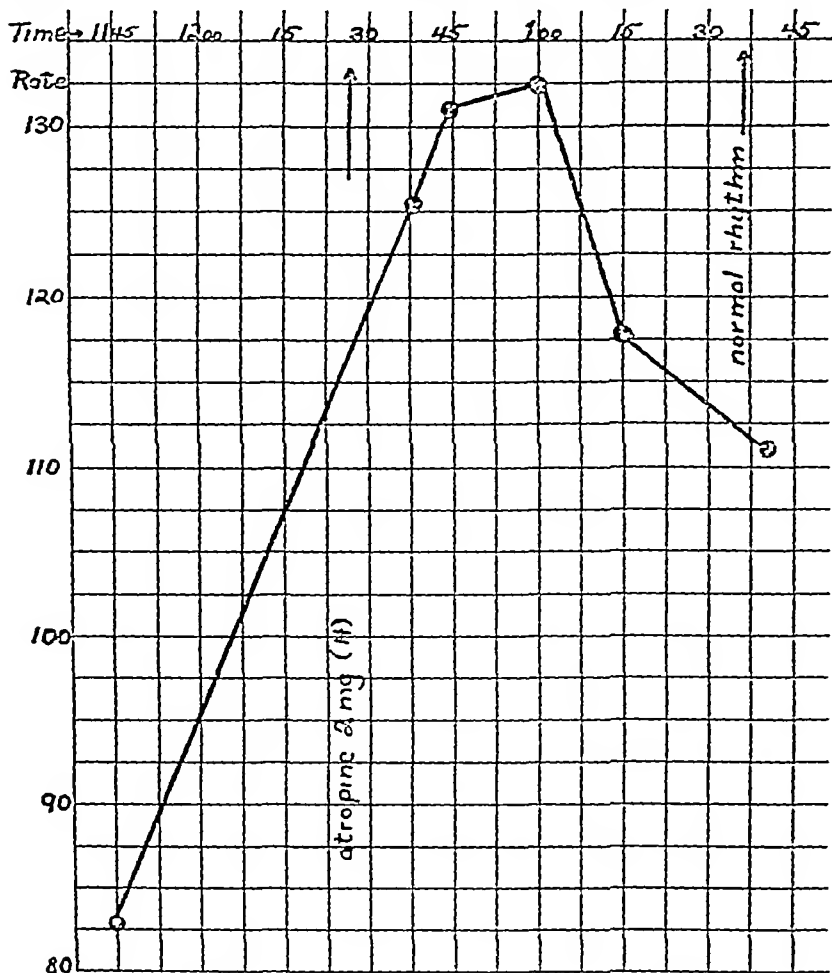


FIG 3 CASE 7

This chart shows the effect of the hypodermatic injection of atropine 2 mgm. One hour and eight minutes after the injection the rhythm became normal.

cases 3 and 4. In case 3 atropine 2 mgm given intravenously caused the ventricular rate to rise from 76 to 93. In case 4, following the intramuscular injection of atropine 2 mgm, the ventricular rate rose from 71 to 107.



Cases 7 was a woman with syphilitic heart disease, who developed auricular fibrillation after the administration of digitalis. On the following day, after the subcutaneous administration of atropine 2 mgm, the ventricular rate rose from 83 to 131, and there was a reversion to normal rhythm one hour and eight minutes after the injection of atropine. The rhythm remained normal thereafter (fig 3)

### DISCUSSION

The following criteria are offered as the basis for judging whether a causal relationship exists between the onset of auricular fibrillation and digitalis therapy

- 1 A history must be obtained that previous attacks of auricular fibrillation were absent. This criterion is admittedly of uncertain value. In cases in which a clear history can be obtained of former transient attacks, there must necessarily be some doubt as to the influence of digitalis in the precipitation of the attack incident to its administration.

- 2 Normal rhythm must be present before digitalis is administered.

- 3 Auricular fibrillation must appear after an effective dose of digitalis as judged by the clinical response and electrocardiographic evidence.

- 4 The abnormal rhythm must persist as long as the use of digitalis is continued in doses sufficiently large to compensate for the elimination of the drug.

- 5 Normal rhythm must be reestablished after cessation of the use of digitalis.

- 6 The changes in rhythm must be clearly established by electrocardiographic records.

- 7 Other factors which tend to bring on transient auricular fibrillation must be eliminated. It is not always possible to be certain that such influences are absent, as periods of auricular fibrillation may occur without any ascertainable cause.

These criteria were met in every instance in the present series of cases. Myocardial failure in itself may be responsible for the appearance of transient auricular fibrillation (Vaquez, 1911), but that this factor was not the direct cause of the onset of the irregularity in the cases described above is shown by the fact that in every instance the irregularity either appeared when the patient was improving, or disappeared when the clinical condition was definitely worse.

No great emphasis is placed upon the figures for the dose calculated according to the body weight or the amounts of digitalis administered, for in the one case, there must be inaccuracy dependent upon variations in susceptibility to the drug and uncertainty regarding the exact weight of the patient, and in the other, the time of onset of fibrillation is unknown and the factor of excretion is variable. The ages of the patients varied from 36 to 58 years. The approximate amount of the drug "active" in the patients, that is to say, the total

TABLE I  
*Clinical Data*

Case	Age	Type of heart disease	Dose of digitalis according to calculated body weight	Amount of digitalis given when fibrillation was detected	Time in days after last dose of digitalis	
					When fibrillation was detected	When reversion to normal mechanism took place
1	48	Cardiovascular hypertensive	grams 1.9	grams* 1.9	2	6
1 (Second admission)				1.9	Same	6
2	36	Cardiovascular hypertensive	1.8	1.6	1	6
3	38	Syphilitic	2.1	†	Same	3
4	40	Syphilitic	1.8	1.5	1	2
5	58	Syphilitic	1.7	1.8	1	2
6	38	Syphilitic	1.8	2.2	Same	2
7	48	Syphilitic	1.4	1.5	1	1½

\* Deduction of 0.15 gram per day for excretion has been made.

† Not known. Patient received digitalis before entering the hospital.

‡ One hour and eight minutes after administration of atropine.

amount actually given minus the amount excreted, was in no instance much over the dose calculated for their body weights. For case 3, no conclusion is drawn because of the previous use of digitalis. The abnormal rhythm was first detected either during the administration of digitalis, or shortly after the drug was withdrawn for some specific reason, such as vomiting, slowing of pulse, the presence of premature beats. Since none of the patients noticed any change in the heart's action, and since a varying period of time elapsed between successive

examinations, it is not possible to judge accurately of the exact time of onset of the irregularity (table 1) Normal rhythm was restored within one to six days after the last dose of digitalis

In no instance did the onset of auricular fibrillation seem to have an appreciable influence upon the patient's condition These observations were made on patients with severe myocardial insufficiency three died (cases 2, 4, 6), one of these of a terminal infection, one (case 5) was practically incapacitated at the time of his discharge from the hospital Of the three who were moderately improved, one (case 1) has returned to the hospital and another (case 7) has died These results are given to illustrate the severity of the degree of heart failure in the patients with whom we are dealing In five of the seven cases, the underlying basis of the cardiac lesion was syphilis Approximately 50 per cent of the colored cardiac patients in this hospital have syphilitic heart disease In view of the relatively small number of cases in which transient auricular fibrillation occurred, too much emphasis is not to be placed on percentage figures It is of some interest to notice, however, that the frequency of transient auricular fibrillation was more than twice as great in patients with, than in those without syphilitic heart disease, in spite of the fact that ordinarily auricular fibrillation is not often associated with this type of heart disease In a series of 363 cases of auricular fibrillation, syphilitic heart disease was present in only 2 per cent (Cohn, 1920)

### *Results with atropine*

It has been shown by Lewis, Drury, Wedd and Ihescu (1921-1922) that in man, atropine 3 mgm given intravenously was usually sufficient to bring about complete vagal paralysis, that a trifle more than 1 mgm given intravenously produced pronounced vagal release, and that 1 to 2 mgm hypodermatically was inadequate to obtain a full effect It was shown, moreover, by a study of the reaction to sufficiently large doses of atropine in digitalized patients with auricular fibrillation, that the effect of digitalis in this condition is the result of both a direct action upon the heart muscle and an action through the vagus nerves In those in whom the ventricular release was slight, the action was predominantly muscular, in those in whom a distinct rise in ventricular rate occurred, the effect was chiefly vagal These

findings were corroborated by the observations upon the auricular rate, for the fall in auricular rate was approximately parallel to the rise in ventricular rate, a finding which is in conformity with the experiments of Lewis, Drury, and Ilescu (1921-1922)

In the present series of observations, the cases may be divided into three types (table 2). In cases 1 and 3 the rise in ventricular rate following large doses of atropine given intravenously was slight. Consequently one may conclude that in these patients digitalis produced its effect chiefly by a direct action on the heart muscle. In the second type, case 7, the response to atropine was greatest. Since this result

TABLE 2  
*Reaction to atropine during the period of auricular fibrillation*

Case	Amount of atropine	Mode of injection	Ventricular rate before atropine	Maximum ventricular rate after atropine	Remarks
	<i>mgm</i>				
1	3*	V†	53	69	Reversion to normal mechanism 1 hour 8 minutes after atropine
	3	V	54	71	
	3	V	60	84	
2	4†	M†	64-68	102	
3	2	V	76-78	93	
4	2	M	71	107	
5					
6					
7	2	H†	83	131	

\* Two milligram followed in 15 minutes by 1 mgm.

† Two milligram followed in 22 minutes by 2 mgm

‡ V = intravenously M = intramuscularly H = hypodermatically

took place after a dose of atropine that was distinctly inadequate, the assumption is that in this patient digitalis acted chiefly through the vagus nerves. This conclusion is suggested by the fact that in this patient reversion to normal mechanism took place after giving atropine. An experimental basis for this judgment exists (Lewis, Drury and Bulger 1921, a). In the third group, cases 4 and 5, the rise in rate was moderate, but considerably less than may be obtained. Since the intramuscular injection is intermediate in effect between intravenous and hypodermatic injection, it may be assumed that in these patients, at least the major part of vagal inhibition was removed

Case 2 in whom there occurred symptoms of atropine poisoning, such as mild mental confusion lasting a few hours, apparently belongs to this group. In this group, then, the action of digitalis was regarded as both muscular and vagal, it is probable that the action on the heart muscle was predominant in case 2. In every instance the general evidences of the action of atropin were present. Unfortunately, counts of the auricular rate are not available, for direct chest leads were not utilized in taking the electrocardiograms (Drury and Ilescu, 1921). Nevertheless, the information derived from a study of the ventricular rate is sufficiently definite to justify the conclusions which have been drawn as to the mode in which digitalis acts.

Only one other instance in man has been encountered in the literature in which the change from auricular fibrillation to the normal mechanism followed the use of atropine (Hering). Since in this patient fibrillation was paroxysmal in type, it is perhaps not quite justifiable to attribute to atropine the reversion to normal rhythm and this is the case also in the present instance. Heitz (1914) gave atropine 1.5 mgm. to several patients with paroxysmal auricular fibrillation, but was never able to cut short an attack. Atropine was similarly unsuccessful in Hewlett's case (1910-1911) of paroxysmal auricular fibrillation.

Experimentally, it has been shown that the induction of auricular fibrillation by faradic stimulation is hindered by the previous use of atropine, and that auricular fibrillation previously established may often be terminated at once by an intravenous injection of this drug (Winterberg, 1908). These results have recently been confirmed and extended by Lewis, Drury and Bulger (1921, a) who showed that the mechanism depended on increasing the duration of the refractory period of heart muscle by removing vagal tone. In case 7 (fig. 3) in which the reversion to normal rhythm occurred slightly more than an hour after the injection of atropine, the change took place after the height of the atropine effect, as judged by the ventricular rate, had been passed. It does not necessarily follow, however, that the atropine effect in the auricles was declining, for curves may be seen (Lewis, Drury, Wedd and Ilescu, 1921-1922) in which the auricular effect outlasts the ventricular.

Comment should be made on the result in the second atropine test of case 1 (fig 1). It was concluded from the small maximum rise in ventricular rate, that the action of digitalis was almost solely muscular. Immediately following the intravenous injection of atropine, the rate rose from 54 to 71. Sixteen minutes later it fell to 48, and then rose again to 68. It is possible that this drop is merely apparent, and is due to the relatively short period of time in which the ventricular rate was counted, as the electrocardiographic record in this instance represented 10 seconds.

There is an alternative explanation, however, since it has been shown (Lewis, Drury, Wedd and Iliescu, 1921-1922) that under certain conditions following the injection of strophanthin, which acts almost entirely directly upon the muscle of the dog's auricle, atropine may increase rather than decrease auriculo-ventricular block and bring about a fall in ventricular rate. Somewhat similar reactions to the administration of atropine, that is to say a fall occurring shortly after the initial rise, followed by a second increase in ventricular rate were seen in the same patient on two other occasions, and also in case 4 (fig 2) in which instance there can be no question concerning the genuineness of the drop in rate, for it was present in a number of successive records.

#### *Mechanism of production of auricular fibrillation by digitalis*

It is not difficult to reconcile with experimental data the appearance of auricular fibrillation in the patient (case 7) in whom digitalis acted chiefly, or solely upon the vagus nerves. It has been demonstrated by a number of observers (McWilliam, 1887, Cushny, 1911, Robinson, 1913, Lewis, Drury and Bulger, 1921, b, Garrey, 1924) that vagal stimulation tends to favor the development of auricular fibrillation. Occasionally, vagal stimulation alone has served to bring on this rhythm. Clinically, the occurrence of paroxysms of auricular fibrillation brought on apparently by purely emotional causes (Heitz, 1914) adds support to the conclusion that in man, under some circumstances at least, strong stimulation of the vagus nerves may cause auricular fibrillation.

It is more difficult to explain the mechanism whereby digitalis, by its direct muscular action, causes the onset of auricular fibrillation.

It was shown by Lewis and his co-workers (1918-1920, 1921) that as a result of the strain placed upon the auricle by a rapid rate of beating, partial refractoriness and its concomitant depression of conduction through the musculature may develop to such an extent that a circus movement, which is now known to underlie flutter and fibrillation, appears. It was further demonstrated (Lewis, Drury and Iliescu, 1921-1922) that the action of strophanthin on the dog's auricle is direct upon the myocardium. When, after administration of the drug, the auricle is stimulated rapidly, partial refractoriness may appear, at the same time, however, the absolute refractory period is increased, an effect which hinders the development of a circus movement (Lewis, Drury and Bulger, 1921, a, Lewis, Drury, Wedd and Iliescu, 1921-1922). On the basis of these experimental observations on the action of strophanthin, it is difficult to understand how digitalis, by its direct action on the myocardium alone, can induce auricular fibrillation.

It seems clear, nevertheless, that in some of our cases auricular fibrillation was induced by digitalis acting almost entirely directly on the myocardium. That is to say, digitalis appeared to bring on this irregularity, whereas under experimental conditions, the injection of strophanthin tends to have the opposite effect. Consequently, some other factor must be sought, the action of which favors the onset of fibrillation in the digitalized heart. It has been pointed out that in all patients in whom the abnormal rhythm developed, there was a serious degree of heart failure. All the changes leading up to, and resulting from this disturbance are not known. At least one, anoxemia, we know to be practically constant. It was shown by de Boer (1921), working with anemic frogs' hearts, in which nutritional disturbances including anoxemia were probably present, that properly timed stimuli produced the onset of fibrillation. It is conceivable that the changes which occur in heart muscle during the course of heart failure are equivalent to those resulting from the high rate which in experiments brings on partial refractoriness and so in turn auricular fibrillation. The muscle under these circumstances may perhaps become altered so that the effect of giving digitalis is to precipitate auricular fibrillation *without* the presence of a high rate. The suggestion made here is in short that auricular fibrillation may occur under

unusual circumstances (*a*) as the result of giving digitalis although ordinarily this drug increases the duration of the refractory period and (*b*) as the result of a state, such as heart failure, which is regarded as equivalent to that in which partial refractoriness, when the rate of beating is high, disposes the muscle to this irregularity. Bases for considering both these possibilities exist in the work of Lewis and others (Lewis, 1918-1920, 1921, Lewis, Drury and Bulger, 1921, a, Lewis, Drury, Wedd and Ilescu, 1921-1922).

The colored patients who served as the basis of this study were, when admitted to the hospital, seriously ill with syphilitic heart disease. It is to the severity of the degree of myocardial failure as the predisposing cause rather than to the etiology that the frequency of transient auricular fibrillation in patients with syphilitic heart disease in this series is attributed.

#### SUMMARY AND CONCLUSIONS

1 A group of seven patients is described in whom the appearance of transient auricular fibrillation seems directly related to the administration of digitalis. The abnormal mechanism appeared during or shortly after the administration of effective doses of a potent preparation of digitalis. The rhythm became normal a few days after the withdrawal of the drug. The presence of fibrillation during the short period of time seemed to have no appreciably harmful influence upon the patients' course. In every instance fibrillation developed in patients with severe myocardial failure. In five of the seven cases, the underlying etiological factor in the disease of the heart was syphilis, and the relationship between this type of heart disease and transient auricular fibrillation due to digitalis is discussed.

2 In most of the patients the response to atropine was observed after the onset of fibrillation. It was concluded that auricular fibrillation following digitalis was due in some cases to strong stimulation of the vagus nerves, but that in most instances it was due to direct action upon the heart muscle. In one patient there was a reversion to normal rhythm following the use of atropine.

3 Myocardial failure is an important, probably necessary, predisposing factor in the production of transient auricular fibrillation by direct action, and possibly by the vagal action, of digitalis.



I wish to express my thanks to Dr E P Carter and Dr F R Dieu-aide for their helpful suggestions during these observations and in the preparation of this paper

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# THE LEUCOCYTE CURVE AS AN INDEX OF THE INFECTION IN RHEUMATIC FEVER<sup>1</sup>

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## INTRODUCTION

The clinical picture of rheumatic fever is so profoundly altered by the suppression of its most characteristic symptoms fever and polyarthritis, following the administration of anti-rheumatic drugs, that adequate consideration is too seldom given to the true course and duration of the infection as it would proceed uninfluenced by anti-symptomatic medication. Any information therefore, which supplements the clinical picture helps towards an understanding of the actual course and duration of the disease. This study was undertaken with the object of determining whether the leucocyte curve might furnish such information, as leucocytosis is a well established sign of the presence of certain infections.

The older clinicians, who studied rheumatic fever before the introduction of salicylate therapy, recognized that the disease might be self-limited and of short duration or might run a subacute or chronic course. Friedlander (1), in a study made over 50 years ago of temperature curves and clinical signs in patients receiving no drugs, divided the disease into three types which he called the monocyclic, the polycyclic and the continuous forms. A chart summarized by us from his published records (fig. 1) illustrates these three types.

## METHODS AND MATERIALS

During the present investigation the leucocyte determinations were made at frequent intervals over a long period of time, those made at each follow-up examination have been used in verifying the normal

<sup>1</sup> Presented before the American Society for Clinical Investigation May 5, 1924

count of each patient. The technique was standardized in order to reduce the experimental error to a minimum. With few exceptions the counts were done by the same individual, each patient's blood was always drawn into the same previously numbered pipette either from a finger or the lobe of an ear. The blood of patients in the hospital was obtained at approximately the same time of day, either between 11 and 12 in the morning, or 3 and 4 in the afternoon. On

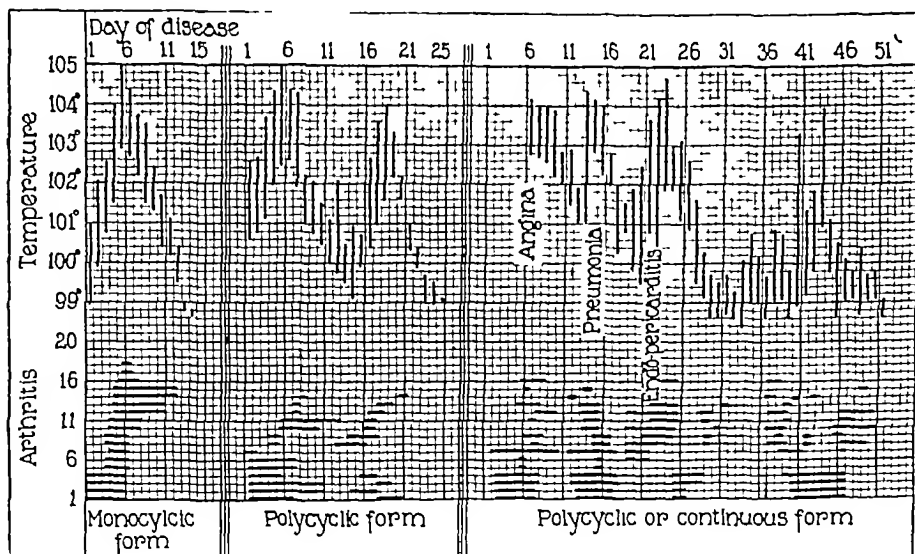


FIG 1 EVOLUTION OF THE THREE TYPES OF RHEUMATIC FEVER UNINFLUENCED BY DRUGS (FRIEDLANDER)

In this and subsequent clinical charts 1 day is represented by a vertical line, the upper and lower limits of the temperature lines represent the range of temperature for the day. Each inflamed joint is represented by a horizontal line, the duration of arthritis in a single joint by the length of the line, and the severity by the thickness of the line. Two or more lines occurring at the same level indicate that the joint was involved more than once.

patients returning for follow-up examination the specimens were collected after an hour's rest in bed. During the clinical study of each patient careful search was made for the presence of non-rheumatic infections in order to eliminate the possibility of their influencing the leucocyte curve.

Altogether the leucocyte curves of 58 patients with active rheumatic fever have been studied. 39 begun during the years 1922 and 1923, and 19 begun during the winter and spring of 1923-24. Because of

the short time elapsing since the onset of the illness of the patients seen during the past year, it has not been possible to determine the normal count in most instances. Averages made of two groups studied during this time have been compared with averages of similar groups followed for 2 years. Some good illustrative cases recently seen have been selected for the present paper. Of the 39 patients followed for the longer period, 9 have been eliminated because of concomitant complications, or nonspecific protein therapy which would have altered the leucocyte picture. A group of 2 additional patients with chorea minor was not considered large enough to incorporate in this report.

The remaining 28 patients followed since 1922 or 1923 and included in this study may be divided into three groups.

I Nine patients with severe polyarthritis who recovered without relapse. This group may be called the *monocyclic* group.

II Nine patients with severe polyarthritis who suffered one or more relapses—the *polycyclic* group.

III Ten patients, mostly children, in whom the predominant feature was the cardiac involvement, one-half of them had subcutaneous nodules. Most of this group belong to the *continuous* type of the disease.

## RESULTS

The exposition of the results obtained and the comparison of the leucocyte curves in the three groups of cases may be facilitated by introducing one or two examples of each group.

### *Group I Monocyclic type (fig 2)*

Case No 1 (No 5),<sup>2</sup> L V, male, 21 years. Admitted January 18, 1923, on the 2nd day of his first attack of the disease, with fever, rapid pulse, severe migratory polyarthritis, a soft blowing apical systolic murmur and moderate leucocytosis. Symptoms increased until the 6th day of illness rapidly diminished under neocinchophen and recovery occurred without relapse. With the disappearance of fever, tachycardia and arthritis, the leucocyte curve fell steadily to normal. No recurrence of illness followed his discharge February 15, 1923, and 1 year later the cardiac sounds were quite normal.

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<sup>2</sup> No 5 refers to case number in composite chart 3.

This case, belonging to the monocyclic type, is typical of all in this group

Figure 3 is a composite chart of the leucocyte curves of the 9 cases belonging to Group I. Two of the 9 patients did not receive anti-

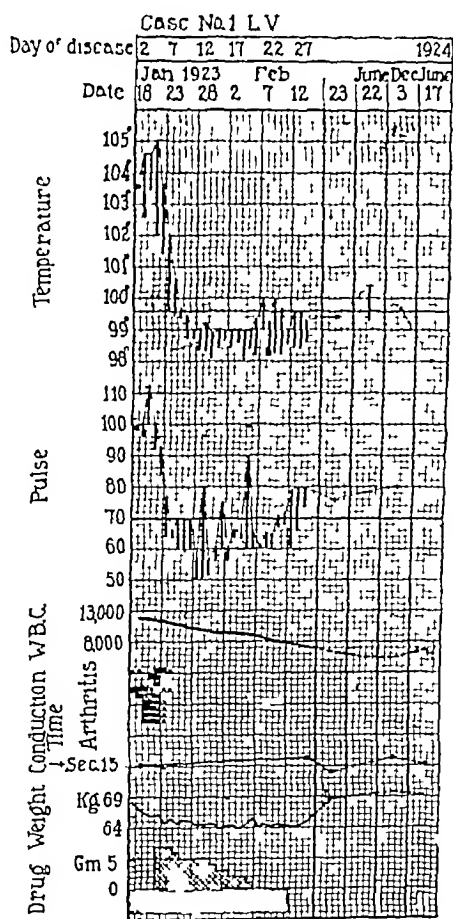


FIG. 2 CASE OF MONOCYCLIC RHEUMATIC POLYARTHRITIS

Here range of pulse for a day is also indicated by limits in pulse line, severe arthritis by heavy solid line, mild arthritis by a dot. Conduction time measured from electrocardiogram taken on day indicated. The dotted lines on the right side of the chart show that the curves were reconstructed from "follow up" examinations.

rheumatic drugs during their attack. In this, as in all of the composite charts, post-operative (post tonsillectomy) counts have been eliminated.

A striking similarity in certain features of all of the curves is noted. There is a rapid and marked drop of leucocytes to normal, or almost normal, following the institution of anti-rheumatic drug therapy and only a slight temporary rise when the drugs were discontinued. This is in marked contrast to the curves of the other two groups.

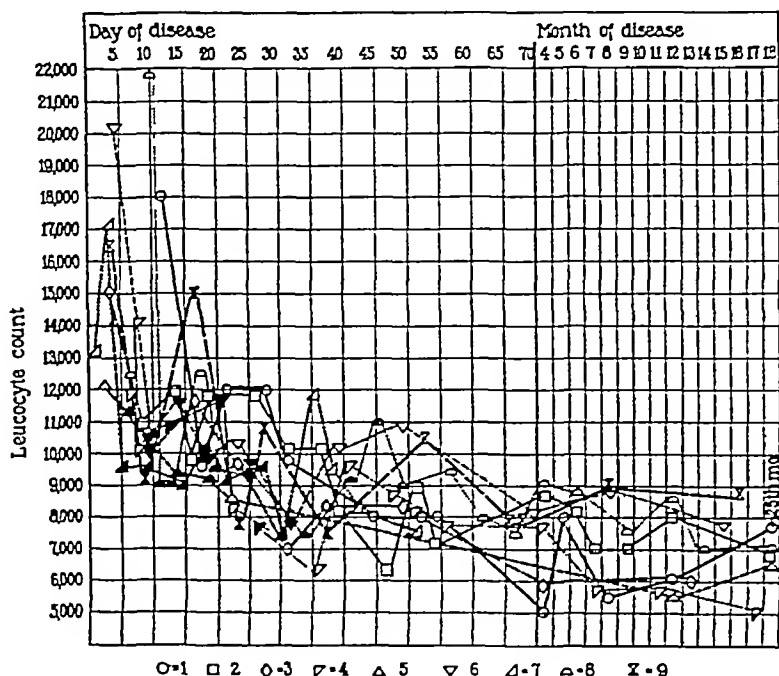


FIG 3 COMPOSITE LEUCOCYTE CURVES OF MONOCYCLIC RHEUMATIC POLYARTHRITIS

Each geometric figure represents a single case. Outline figures indicate patient was not under influence of drugs, solid figures that patient was under drug therapy.

### *Group II Polycyclic type*

This included 9 adult patients in whom the outstanding clinical manifestation was polyarthritis, but in whom there was also evidence of cardiac involvement, all suffered one or more relapses.

Case No 2 (No 16), B R, female, unmarried, age 17. Admitted March 26, 1922, on the 14th day of her first attack of rheumatic fever with severe, extensive polyarthritis, high fever and rapid pulse, marked prolongation of conduction time.



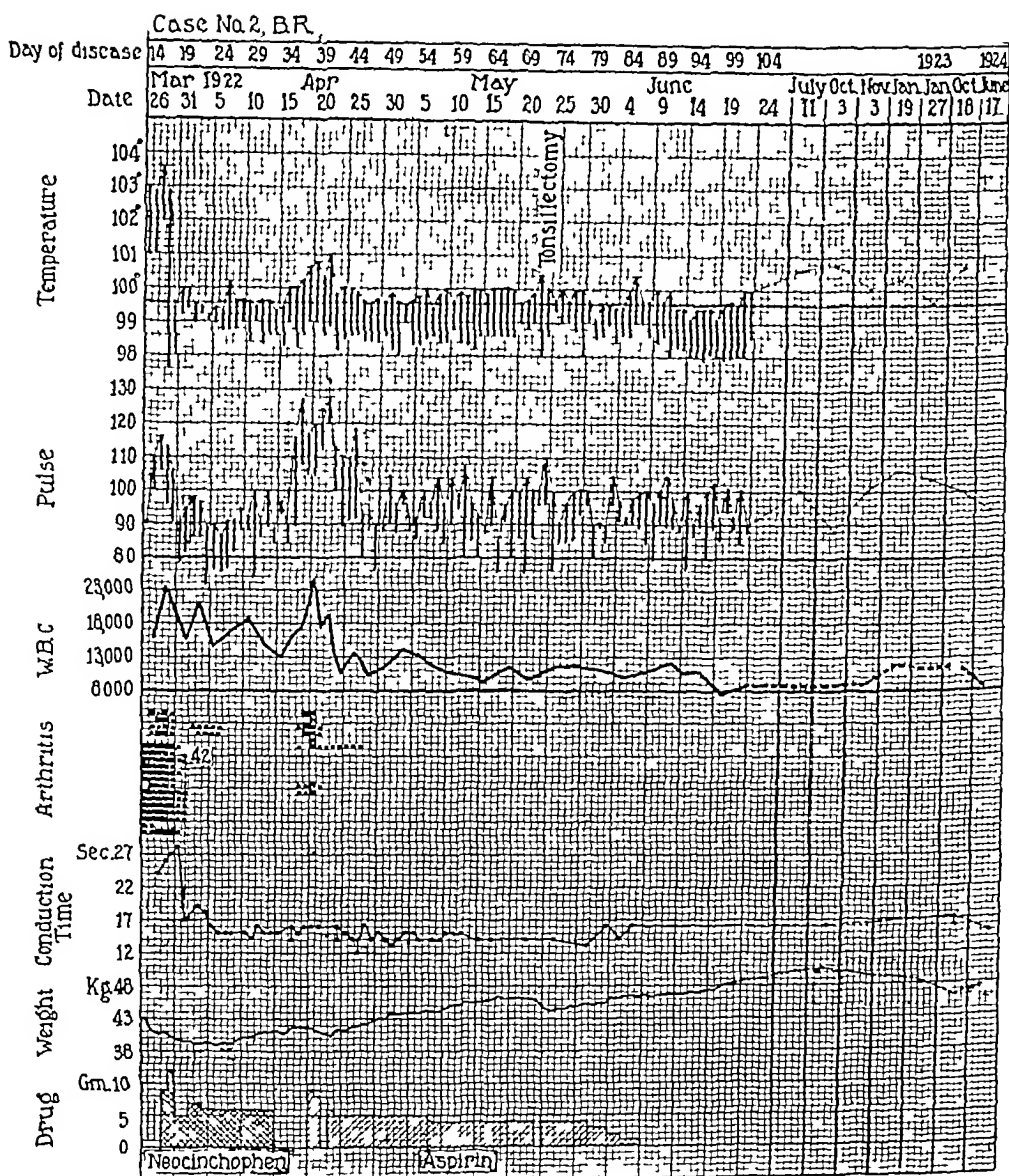


FIG 4 RELAPSING OR POLYCYCLIC RHEUMATIC POLYARTHRITIS

Vertical dotted lines in pulse curve indicate paroxysms of tachycardia Vertical dotted line, April 1, indicates P-R time of 0.42 seconds

in electrocardiogram, and leucocytosis. Under neocinchophen therapy all symptoms disappeared except leucocytosis. After 17 days of medication the drug was withdrawn, this was followed promptly by a relapse in which rapid pulse and paroxysms of tachycardia, together with polyarthritis, were marked features, these symptoms were relieved with aspirin. Tonsillectomy on the 73rd day of the disease. The discontinuance of aspirin on the 88th day was followed by recovery without relapse. The leucocyte count became practically normal on the 100th day. Discharged June 23, 1922. Two years later there were no auscultatory signs of cardiac disease.

This case illustrates very well the prognostic value of the leucocyte count. During the 4th week of her disease, while she was still receiving neocinchophen, her temperature, pulse, and conduction time were normal, and there was no evidence of arthritis, she was gaining weight and feeling well enough to beg to be allowed up. In fact, the only sign of persisting infection was continued leucocytosis. That the disease was not yet terminated but only rendered symptom-free by the anti-rheumatic medication was proved by the relapse which promptly followed discontinuance of the drug.

Case No. 3, M C, female, married, age 35. Admitted November 1, 1923, on the 8th day of her first attack of rheumatic fever with severe, extensive polyarthritis, fever, rapid pulse, a systolic murmur, and a moderate leucocytosis. Under full therapeutic doses of sodium salicylate the symptoms promptly disappeared and the white count fell to 9000. Discontinuance of sodium salicylate on the 34th day of disease was followed by a relapse with fever and extensive polyarthritis. These symptoms cleared up under treatment with maximal doses of neocinchophen. On the 43rd day the leucocyte count fell to 4700, on this day the temperature was subnormal, ranging from 98° to 98.7°F (rectal). The white count rose 4 days later to 11,400 and continued to rise for another week. Tonsillectomy on the 55th day followed by a leucocytosis of 24,000 (recorded by dotted line). Neocinchophen discontinued on the 65th day. Steady improvement occurred and the leucocyte curve fell gradually to normal which was below 8000, no relapses followed. Discharged January 10, 1924, on the 79th day of disease. Since that time she has been quite well. The apical systolic murmur is still present, 6 months after discharge.

This patient is an example of the polycyclic type where polyarthritis was the outstanding feature. The leucocyte count remained above 8000 throughout the period of salicylate medication. This level was not normal for this patient as was proved by counts made during convalescence and at subsequent follow-up examinations.

That this low-grade leucocytosis indicated a persistence of the rheumatic disease was shown by the relapse which quickly followed the discontinuance of anti-rheumatic medication. The drug, therefore, had not eliminated the infection but had merely held in check its

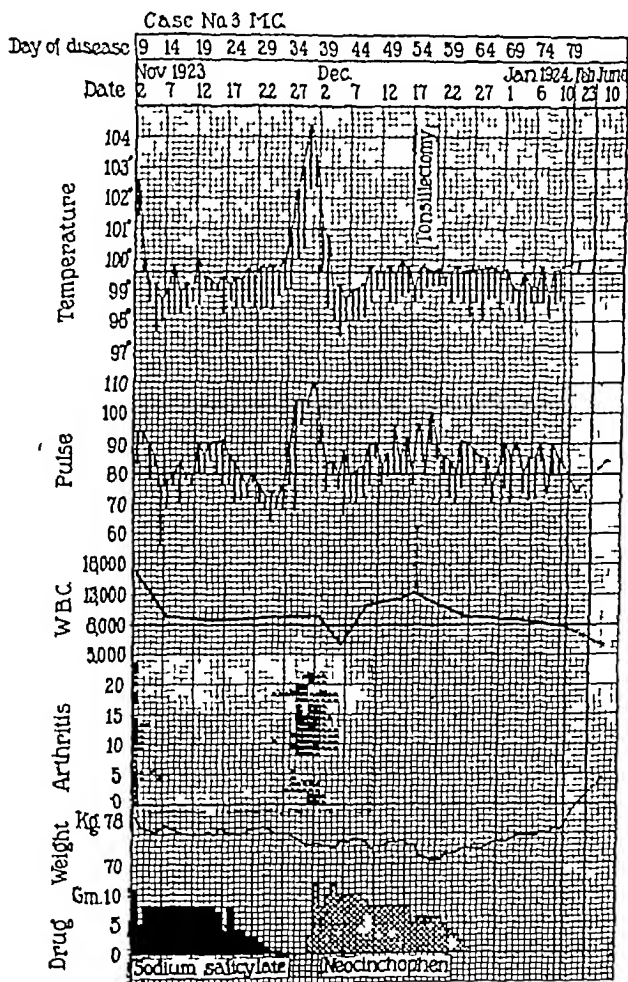


FIG 5 RELAPSING OR POLYCYCLIC RHEUMATIC POLYARTHRITIS

Vertical dotted line on W B C indicates post-tonsillectomy leucocytosis

clinical manifestations. The very low count (4700) which followed the administration of large doses of neocinchophen (10 to 12 grams a day) deserves comment. It occurred during a short period of sub-normal temperature following a high fever of  $104.4^{\circ}$ , it is interesting that it took place in the relapse and not in the initial attack

In several instances a similar temporary fall of the leucocytes below normal was observed following the onset of anti-rheumatic medication

This case is not included in the composite curves because the leucocyte curve has not been followed for a year

Figure 6 is a composite chart of the leucocyte curves of the 9 cases in the polycyclic polyarthritic group In Cases Nos 10 and 11 no

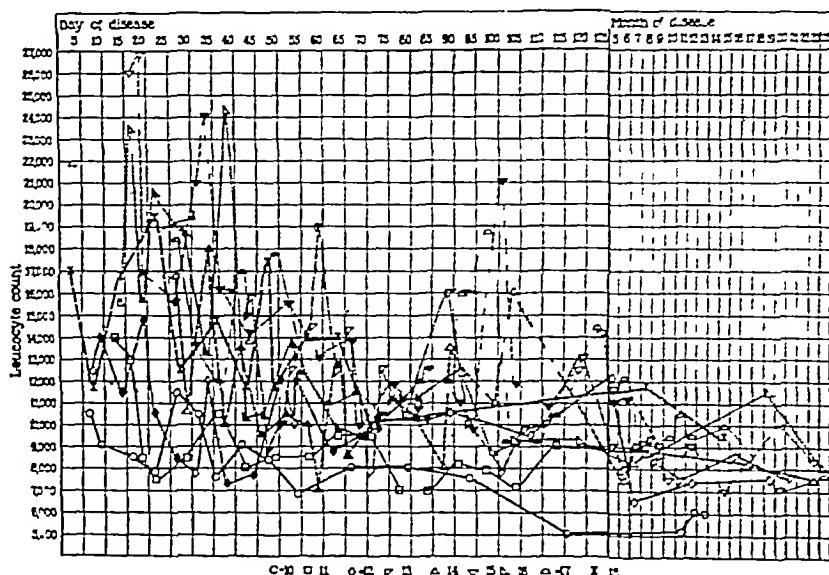


FIG 6 COMPOSITE LELCOCYTE CURVES OF POLYCYCLIC RHEUMATIC POLYARTHRITIS

drugs were administered The curves in all instances showed a tendency to remain above normal for a much longer period than those of Group I The effect of drug therapy was also less marked Following the institution of treatment the fall towards normal was less noticeable, and in several instances, even though the drug was continued, there was a marked rise following an initial fall In all cases except No 15 there was a rise of from 4000 to 9000 following the discontinuance of medication, in case 15 there were two rises of 8000 and 11,000 respectively, even though the patient was under the influence of drug therapy

The difference in response to drug therapy of the leucocyte curves of patients in the two groups is brought out more strikingly in figure 7. Here a larger number of cases are included in each group, but the estimations are dated from the first day of treatment, and the curves are charted for only 35 days. The upper and lower limits of each group are indicated in the upper part of the chart, and the average of each group by the heavy lines in the lower part. It is evident

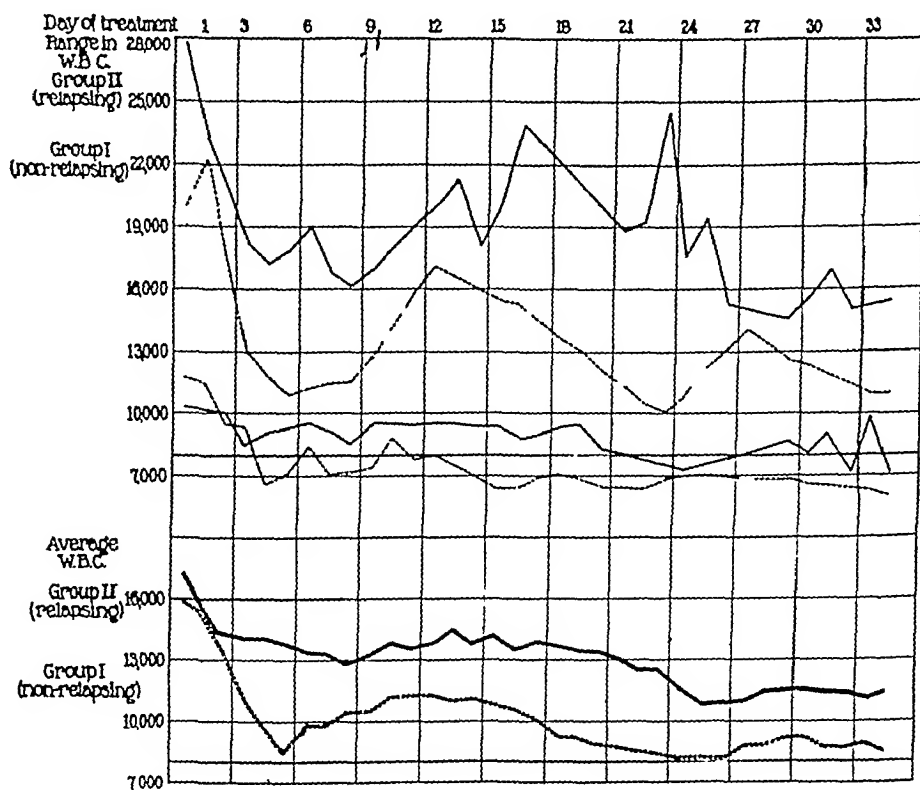


FIG 7 EFFECT OF MEDICATION ON THE LEUCOCYTE COUNTS IN RELAPSING AND NON-RELAPSING TYPES OF RHEUMATIC FEVER

that in this 5 week period the leucocyte curve of those patients who did not suffer a relapse was much more rapidly and profoundly influenced by drugs than was the curve of those who had relapses

### *Group III Continuous form*

In this group have been included those patients in whom the clinical manifestations were chiefly cardiac and those patients who

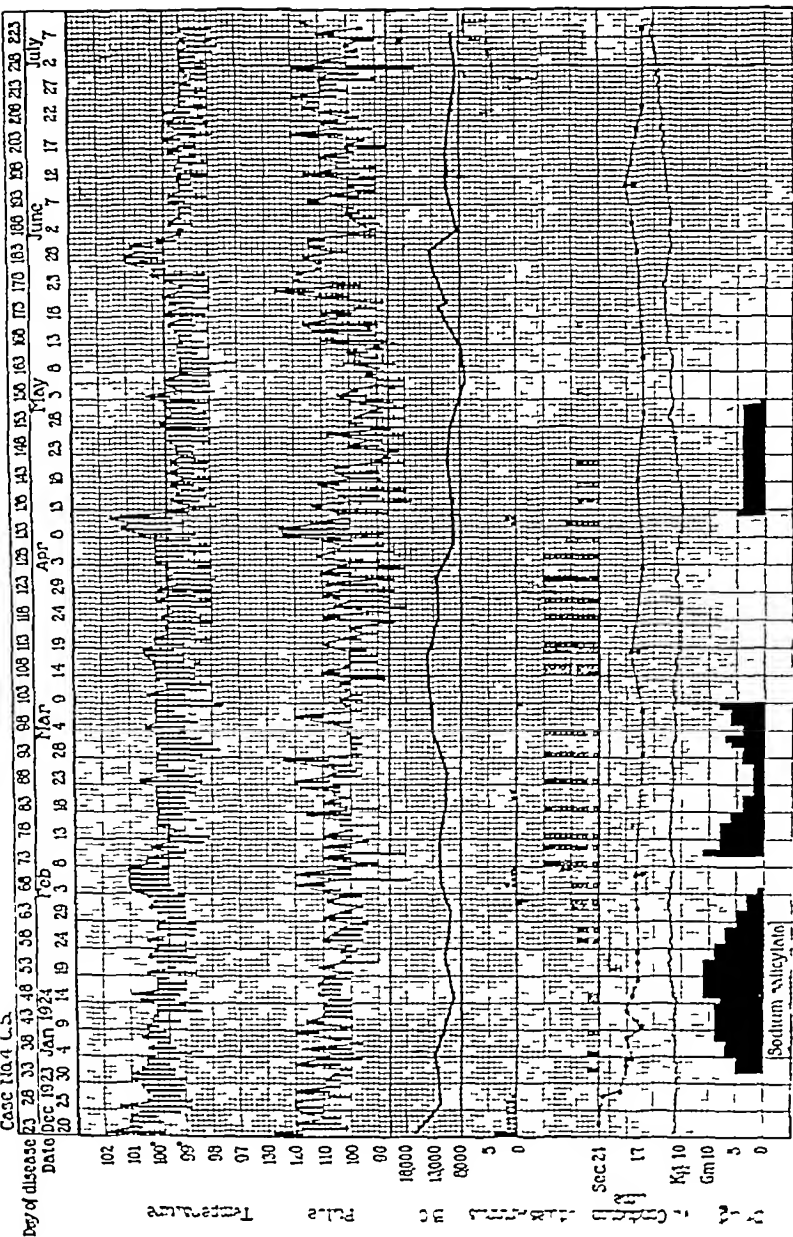


Fig. 8. CONTINUOUS OR POLYCYCLIC FORM OF RHEUMATIC FEVER

(arthritis) and subcutaneous nodules predominating symptoms that old nodules previously found in same location are still present. Solid circles indicate new nodules, rings indicate

had subcutaneous rheumatic nodules, with the exception of two young adults, all were children

Case No 4, C S, female, 12 years of age, was a patient in this hospital for 4 months in 1920 with rheumatic fever and has been under observation ever since. Readmitted December 19, 1923, on the 22nd day of her 3rd attack of rheumatic fever with pyrexia, mild polyarthritis, tachycardia, prolongation of conduction time to 0.24 second, and a leucocytosis of 16,400. Within 2 weeks subcutaneous rheumatic nodules began to appear and continued to do so for over 3 months. Occasional mild arthritis. Three courses of sodium salicylate were given, in two of which the drug was pushed to 11 grams per day, the limit of tolerance, with little, if any, influence on the leucocyte curve. During her stay in the hospital, in addition to a fairly continuous low grade fever for 4 months, there were several distinct cycles of higher temperature and more rapid heart action. The leucocytosis persisted until the beginning of the 5th month when the count fell to normal for a week and then preceding the last bout of fever had a slow rise to 13,000. After the temperature had returned to normal the leucocytosis again subsided, but the leucocytes remained slightly above normal at the time of discharge.

This case illustrates both the continuous and relapsing nature of the disease and the persistence of leucocytosis, as well as the slight, or negligible influence of anti-rheumatic drugs on the leucocytosis during the first 4 months. An interesting point is the transient fall of the leucocyte curve to normal in the 5th month, and its subsequent rise which preceded the last cycle of fever and tachycardia. In several other patients a relapse was heralded by a similar rise in the leucocyte count. It shows that one or even two normal counts are not conclusive evidence of the termination of infection, but that counts must be made frequently enough, and over a sufficient period of time, to set forth the trend of the leucocyte curve.

This case is not included in the composite curves because the leucocyte counts have not been followed for an entire year.

Figure 9 is a composite chart of 10 cases, all had definite cardiac involvement, slight or no arthritis, and Nos 24 to 28 had subcutaneous nodules, which are well known indicators of severe types of rheumatic infection. In many of these patients it was difficult to determine the time of onset of the infection, in two, Nos 22 and 28, there was a history of illness of 1 to 3 years, but the curves are charted with the first week as the beginning of the last exacerbation. Because of the mildness of arthritis one-half of these patients did

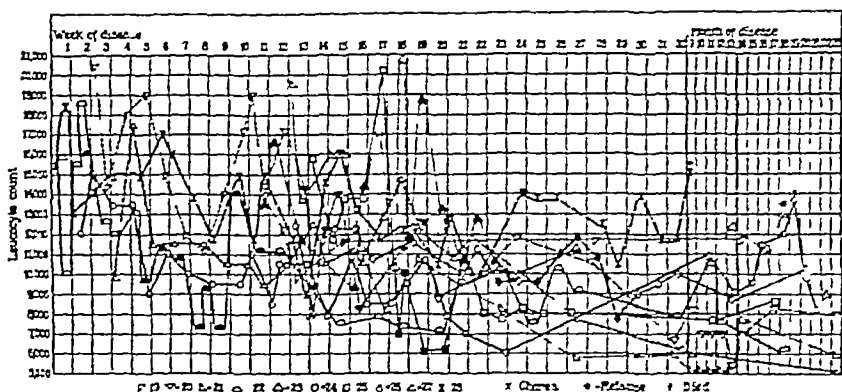


FIG 9 COMPOSITE LEUCOCYTE CURVES OF CONTINUOUS OR RELAPSING RHEUMATIC FEVER, CARDITIS AND SUBCUTANEOUS NODULES PREDOMINANT SYMPTOMS

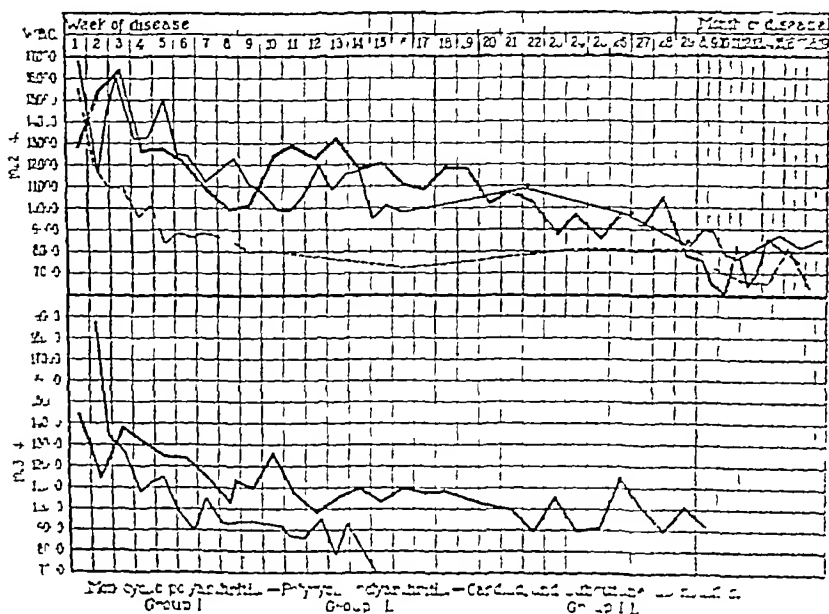


FIG 10 AVERAGE LEUCOCYTE CURVES OF THE THREE DIFFERENT GROUPS FOLLOWED 1922 TO 1924 AND OF GROUPS II AND III FOLLOWED 1923-24



not receive anti-rheumatic drugs. Among those treated, except No 25, there was the same lack of permanent response as was previously noted in Group II. In the one fatal case, No 28, leucocytosis was always present, temporary improvement during the middle third of her stay in the hospital was accompanied by a lower level, and the final failure was preceded and accompanied by a rise in the curve.

In the upper part of figure 10 is given the average curve of the three groups, followed during the years 1922-23-24 and in the lower part, for comparison, similar averages of patients in Groups II and III first seen in 1923-24. Because of the shorter period of observation of these cases it has been impossible to extend the curves as far as those begun in the earlier years, but they are useful in corroborating the first series of observations. In Groups I and II of the upper curves the averages after the 16th week represent compilations of counts made within 2 weeks of these points respectively, this was necessary because the number counted in any one week in this late period was too small for compiling averages.

Several striking features are brought out by these averages. First, the initial and almost parallel fall in all five curves up to the 6th to 8th week. The monocyclic group then became and remained normal. Second, the subsequent rise and fall, more or less wave-like, of the other curves. Group II of 1922-24 showed a marked fall in the 15th week, with a subsequent rise, while the comparable group of 1923-24 showed a drop to normal at this time, whether there will be a later upward trend in this last group cannot now be stated. The curves of Group III in both years tend to parallel one another in a striking manner, and in general indicate that the patients with marked clinical evidence of carditis had the most prolonged leucocytosis.

#### DIFFERENTIAL FORMULA

Differential counts were made in 13 patients. The leucocytosis was shown to be caused chiefly by an increase in the polymorphonuclear neutrophiles. A low grade eosinophilia occurred in only 2 patients. Both had chorea, during the course of their disease, 1 in quite severe form. In these the eosinophiles varied from 2.5 to 5.7 per cent and 1.5 to 4, respectively. In passing it should be mentioned that 1 other patient with chorea also had an eosinophilia.

## RELATION OF LEUCOCYTE CURVE TO RESIDUAL VALVULAR DISEASE

The leucocyte curves of these cases have been analyzed with reference to the development of chronic valvular lesions, in an attempt to find some factor which would assist in prognosticating this important sequel of rheumatic fever. While no correlation has been established between the configuration of the curves and the subsequent evidence of residual valvular disease, the interesting fact was brought to light that at the end of  $1\frac{1}{2}$  to 2 years cardiac murmurs were audible in only one third of the patients having monocyclic curves, in two thirds of those with polycyclic polyarthritis and in all falling in Group III. It must be mentioned that murmurs were present in all of the patients during their stay in the hospital. Of the 6 patients in the monocyclic group in whom the murmurs disappeared 2 each had had two previous attacks of rheumatic fever the rest were suffering from their first attack. In the polycyclic polyarthritis group 1 of the 3 patients in whom the murmurs disappeared had had one attack, the other two had not had previous attacks. The differences between the groups with respect to residual heart murmurs indicate that in the cases studied the incidence of valvular disease was much higher in the case which ran a chronic course with a consequent prolonged period of leucocytosis.

## DISCUSSION

While it is well known that leucocytosis is a feature of the early stages of rheumatic fever, and casual observations indicate that there is usually a drop in the curve when fever diminishes either spontaneously or as a result of antipyretic drugs, except for the report of a few patients by Takeno (2) and a still more complete study by Korowicki (3), we have been unable to find any studies in which the leucocyte curve has been used as an index of severity or persistence of infection in this disease. In comparing the clinical symptoms and leucocyte curves in a fairly large series of patients we have found that in the majority of instances these curves have considerable value. It is true that they have no greater absolute value than any other sign in clinical medicine, and that they are of most use when taken together with other features of the disease. But, in general

it may be stated that in the absence of evidences of concomitant non-rheumatic infection, a persisting leucocytosis signified persistence of rheumatic infection, and conversely that repeated normal counts indicated that the attack was drawing to a close. The latter statement was invariably true if the patient was free from the influence of medication. In other words, the administration of anti-rheumatic drugs—sodium salicylate, aspirin, neocinchophen and the ethyl ester of phenylcinchonic acid, all of which acted in essentially the same manner—was often accompanied by a fall in the leucocyte curve, sometimes to normal. But whenever the discontinuance of the drug was followed by a count steadily rising to 4000 or more above the previous level a relapse ensued, proving that the rheumatic infection was not yet terminated. And, conversely, when the leucocyte curve remained normal after the discontinuance of the drug, or rose for a few days and then fell again to normal, no relapse occurred and the patient went on to recovery.<sup>3</sup>

This apparent depression of leucocytosis by drug therapy is of considerable interest. By following through the course of individual curves on the composite charts it will be seen that it occurred in a number of instances. When the infection was still active, however, the depression to normal was rarely complete, that is to say, the count rarely fell to normal during medication in those patients with the relapsing type of the disease, and when it did it rose again after the drug was withdrawn. In this group of patients, moreover, there was frequently a rise in the leucocyte curve even while the individual was under the influence of drugs. The mechanism of drug depression of leucocytosis is not understood. It may be either a direct effect of the drug on the hematopoietic system, or a result of the suppression of the exudative inflammatory phenomena, e.g., of the arthritis, which is stimulating leucocytosis. In this connection it is interesting to note that the fall in leucocytes induced by drugs was greater and more rapid in patients with extensive polyarthritis than in those in whom the chief feature was carditis or carditis and subcutaneous

<sup>3</sup> We have recently seen a patient who during the 5th month of his disease had a count ranging between 7500 and 8500. Persistence of low grade infection, however, was indicated by a daily temperature of 100°F and the appearance of a new crop of subcutaneous nodules.

nodules As already mentioned in another place (Swift, 4) we may consider that against the infectious agent of rheumatic fever there are two types of tissue response exudative and proliferative The exudative, of which leucocytosis may be considered an example, is much more subject to the influence of the anti-rheumatic drugs than are the proliferative types, such as subcutaneous nodules

Another noteworthy point is that the leucocyte curves of patients with subcutaneous nodules, rheumatic carditis, or the subacute relapsing type of polyarthritis was more markedly affected by drugs late in the disease than in the earlier months In other words, when the patient had developed a considerable degree of resistance against the infection this was, to a certain extent, made manifest by a depression of leucocytes following the exhibition of drugs which earlier in the disease had little effect on the curve The curves in subacute and chronic cases late in the disease, even though above normal, usually were on a lower level than early This lower trend probably indicates the development of some degree of immunity In considering, therefore, the relation of the leucocyte curve to the type of disease and to treatment, the period of infection, as well as the type of tissue response shown by the patient must be taken into account.

The prognostic value of the leucocyte curve in respect to the development of chronic cardiac valvular disease has already been mentioned It would not be rational to suppose that some one tissue would enjoy special favor in relation to prognosis It is probable that, as a rule, several tissues or organs are affected by the "virus" of rheumatic fever When the infection is severe or prolonged the endocardium is usually affected, when the infection is light or the body resistance is very good the heart usually escapes permanent injury In so far as the leucocyte curves are of help in pointing out the degree of severity or of persistence of infection, they are of value in prognosis concerning the cardiac condition

A question naturally arises as to what should be considered the normal white blood count We feel that 8000 to 9000 should be considered the upper limit of normal, because a fair sized series of non-rheumatic controls, and also several former patients who had completely recovered from their infection, had repeated counts below

this level Several "follow up" patients during the past winter whose leucocyte curves had remained consistently between 10,000 and 12,000, in spite of absence of other symptoms, have suffered from new attacks of rheumatic fever It is highly probable that these attacks were due to recrudescence of latent or almost dormant infections If similar experiences are repeated in a larger group, it is possible that the leucocyte curve may be found one of the most important guides to the type of treatment i e, rest or exercise, of a given individual

The differential formula has not proven to have any prognostic value Only in patients with chorea or with erythema multiforme have we found an eosinophilia, similar findings in chorea are fully reviewed by Berger (5) We have been unable in other forms of rheumatic fever to confirm the possibility suggested by Klinkert (6) that an increase in eosinophiles in many infections might be an indication of a tendency towards recovery In a large group of patients, therefore, we feel that time would be more profitably spent in making total leucocyte estimations each week than in making complete differential counts at longer intervals

As rheumatic fever is considered by most observers to be usually a self-limited disease, and as the time of limitation as well as the amount of permanent damage suffered by the patient is probably dependent to a certain degree upon the type of treatment, it is important to have available every source of information as to the course of the disease And the leucocyte curve is one of value, for it does not require elaborate equipment or great expenditure of time Curves constructed from counts made at regular intervals and under constant conditions are of much greater value than occasional counts made at irregular periods

#### CONCLUSIONS

- 1 Leucocytosis is a concomitant of rheumatic fever
- 2 From leucocyte counts made at frequent intervals and under constant conditions curves can be constructed which give an approximate idea of the severity and duration of the infection
- 3 Patients in whom there are predominant exudative phenomena such as polyarthrits, pleurisy or pericarditis, together with high

fever, usually have more marked leucocytosis than those in whom the tissue reaction is chiefly proliferative such as is seen in myocarditis, endocarditis, or subcutaneous nodules

4 A high leucocyte curve is often depressed when the patient is under the influence of anti-rheumatic drugs Under such conditions if the infection is mild and of short duration the curve approximates normal and remains there, if, on the other hand, the infection is persisting the curve either remains constantly above normal, or tends to rise with the discontinuance of drug therapy

5 Relapses are usually heralded by a rise in the leucocyte curve

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# THE ACTION OF PITUITARY EXTRACT ON THE HEART OF THE UNANESTHETIZED DOG

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## INTRODUCTION

Although the effect of the extract of the posterior lobe of the pituitary gland upon the mammalian heart has been studied by a number of investigators, the results have not been uniform. In the first study of the action of pituitary extracts, Oliver and Schäfer (1895) found that with the vagi intact no inhibition of the heart took place, but that with the vagi cut there was a slight diminution in heart rate. Howell (1898) obtained a definite slowing with the vagi intact. When the vagi were cut or under the influence of atropine, a slowing of cardiac rate of lesser degree was observed, and he attributed the action of the drug to an influence not only on the cardio-inhibitory center but also to an effect on the heart muscle or the intrinsic nervous mechanism. Von Cyon (1898) obtained similar results. Hedbom (1898), Cleghorn (1899), and Dale (1909), using isolated mammalian heart preparations, found that the rate was lowered under the influence of pituitary extracts, indicating that the action was in part at least direct on the myocardium. Schafer and Vincent (1900) observed but little slowing of the rate when the vagi were paralyzed by atropine, and occasionally no fall in rate even with the vagi intact. They concluded, however, on the basis of the experiments of Howell and of Cleghorn, that in addition to the vagal action, there must be a direct effect on the cardiac muscle. Garnier and Thaon (1906) ascribed the action wholly to an influence through the vagi, for no slowing occurred after these nerves were sectioned. Wiggers (1911) obtained slowing upon perfusion of the drug through the isolated heart, while with the heart in situ, a variety of results was seen. In



some instances no slowing took place, in others the slowing was immediate, while in other cases the slowing was gradual in its onset. As a result of his experiments he formulated the hypothesis that the early effect was due to stimulation of the cardio-inhibitory center, and the late slowing was due to a direct action on the heart. "Either, both, or neither of these actions may follow the injection of pituitary extract, the factors determining the reaction being unknown." Claude, Porak and Routier (1913) studied the effect of pituitary extracts on the heart of the rabbit utilizing the electrocardiograph. Following administration of the drug, conduction defects and slowing of the heart were seen, with or without section of the vagi. Hecht and Nadel (1913) also used the electrocardiograph and found evidences of a direct action on the heart of unanesthetized animals. However they attributed the slowing chiefly to vagal action. Houssay (1918) as a result of his own and a study of previous experiments, concluded that the effect was in part vagal and in part direct on the myocardium.

It will be seen that although a number of the reports are contradictory, the weight of evidence favors the view that extracts of the pituitary affect the heart not only through stimulation of the cardio-inhibitory center, but also through a direct action on the cardiac muscle.

#### METHODS

We have performed a number of experiments upon three unanesthetized dogs which had been trained to submit to procedures such as we employed. We wish to emphasize the absence of anesthesia in these experiments. Differences between the response of anesthetized and unanesthetized animals to drugs have been observed previously by Kolls and Geiling (1924), and we feel that the constancy of the results which we have obtained is in large measure due to the use of unanesthetized animals. Precautions were taken to avoid factors which might disturb the animals. They were handled by the same attendants, and the surroundings were kept as quiet and uniform as possible. The animals were rested until fairly constant heart rates were obtained before the experiments were started.<sup>1</sup> The drugs were injected into a vein of the foreleg, each

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<sup>1</sup> The resting rates varied on different occasions from 76 to 130. We have learned from Prof. E. K. Marshall, Jr., that with prolonged rest rates of approximately 60 may be obtained. We do not believe, however, that our results are significantly affected by our failure to secure truly basal rates. Before atropine, the slowing following the injection of pituitary extract would have been less striking, while after atropine, the results would have been unchanged.

administration lasting five to ten seconds. The extract of the posterior lobe of the pituitary (Armour's Pituitary Liquid) was given in 1 cc. doses (except in three experiments in which 0.1 and 0.2 cc. were given). Atropine was given in doses of 1 mgm. repeated at about 15 minute intervals, and in some experiments in single doses of 5 or 6 mg. The effects were recorded with the electrocardiograph, lead III being used. Records were taken at intervals of about 15 to 30 seconds in the early periods of the experiments, and at 2 to 5 minute intervals later. In some instances, continuous records were taken during the first few minutes following the injection of the pituitary extract. Only one observation a day was made on any individual animal. The heart rates were calculated on the basis of counts made of six second periods from the electrocardiographic curves (occasionally three second periods when rapid changes were taking place), so that slight changes are somewhat exaggerated. The error in the counts is probably not more than  $\pm 2$  per minute. The P-R intervals were occasionally difficult to measure accurately on account of the form of the curves. At times the onset of the P wave was not sharp, while in other records the P was fused with the preceding T or R waves.

#### EXPERIMENTAL

*Preliminary experiments with atropine.* Several observations were made upon the effect of atropine alone. When given intravenously in doses of 1 to 5 mg., the maximum vagal release was obtained within 45 seconds to one minute. One milligram was often as effective as 5 mg. in procuring a complete vagal paralysis, as judged by the failure to secure a further effect with subsequent injections of the drug in the few instances in which this was done. Our animals G1, G2, G3, weighed 11, 14.5, 9 kilos respectively, so that 1 mg. amounted to 0.09, 0.06, and 0.11 mg. per kilo. Lewis, Drury, Wedd and Iliescu (1921-1922) found that 0.05 to 0.1 mg. of atropine per kilo of body weight were necessary for the complete abolition of vagal tone in anesthetized dogs. However, since the rate tended to be a little higher after 5 or 6 mg. doses than after 1 mg. doses even when this dose amounted to 0.11 mg. per kilo, it is probable that 1 mg. brought about, in some instances a vagal paralysis almost but not entirely complete, and it is further likely that larger doses than those given by Lewis, Drury, Wedd and Iliescu are frequently necessary to procure complete removal of vagal tone in unanesthetized animals.

The full effect of 5 mg. was usually maintained for about 7 to 10 minutes, after which there was a gradual fall in rate. At the end

of 35 to 40 minutes the rate was about 10 to 15 per cent lower than the maximum rate reached. When 1 mg. was used, the fall usually began within 3 to 5 minutes after the highest rate was reached, and at the end of 15 minutes the drop was definite. Table 1 illustrates a more or less typical result.

As repeated doses of 1 mg. were given, the rate usually rose to that obtained originally, but vagal tone was regained more rapidly. The maximum rate varied considerably in the same animal on different

TABLE 1  
*The effect of repeated doses of 1 mg. atropine*  
G 2 Resting rate 80

Atropine	Time	Rate
<i>mg</i>	<i>minutes</i>	
1	1	224
	6	208
	15	184
1*	1	218
	4	206
	14	180
1*	1	208
	4	192
	14	196
1*	1	222
	3	206
	5	194

\* These administrations of atropine were given immediately after the last count given above.

days, thus in one dog, G2, the rates obtained on different days with 1 mg. were 204, 224, 232, 243. It is possible that 1 mg. did not always cause a complete paralysis of vagal inhibition in this animal, for the test of the effect of the further administration of atropine was made on only a few occasions. That an actual variation may exist, however, is shown by the fact that the injection of 4 mg. shortly after the first dose of 1 mg. failed to increase the rate above 232, although this was lower than the peak reached on another occasion,

243 Moreover, at another time, the maximum rate after 5 mg was  
217<sup>2</sup>

Following an injection of atropine, changes in the form of the electrocardiogram were constantly present to a greater or lesser degree. The R wave was lowered, the S wave deepened, and the amplitude of T was increased (figs 1 and 2)

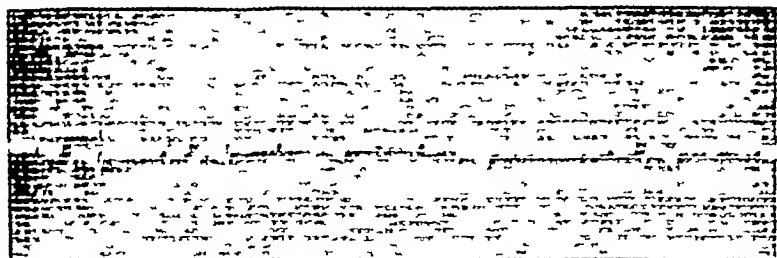


FIG 1 THE NORMAL ELECTROCARDIOGRAM OF THE RESTING DOG (G1)  
Standardization in all records 1 millivolt = 1 cm Time lines = 0.04 seconds

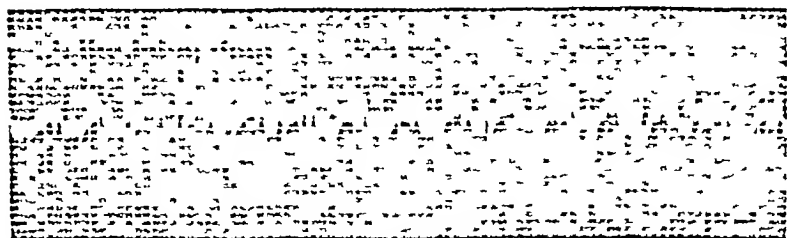


FIG 2 ELECTROCARDIOGRAM TAKEN SHORTLY AFTER THE ADMINISTRATION  
OF 1 MG. ATROPINE (G1)

The normal record of this animal (G3) is similar to that of figure 1

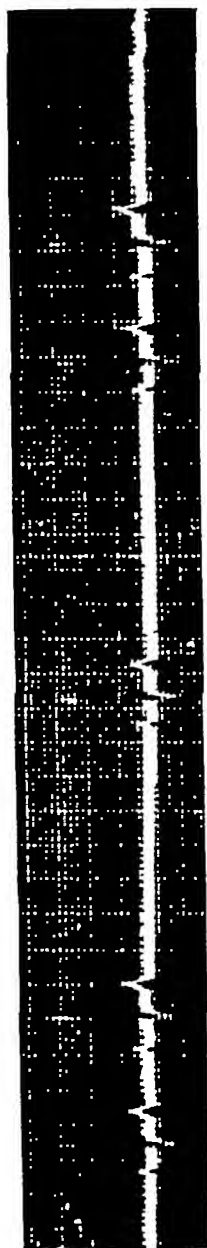
### *The effect of pituitary extract in animals before atropine*

In three experiments 1 cc pituitary extract was given and the results were essentially similar in each instance (table 2). Seven to

<sup>2</sup> It is not likely that variations in the potency of the drug were responsible for these discrepancies, for the atropine solutions were always made from tablets taken from the same bottle throughout the entire series of experiments

ten seconds after the start of the injection there was a sharp rise in rate, lasting 7 to 12 seconds. This rise was associated with a disappearance of the respiratory arrhythmia commonly seen in dogs, and a decrease in the P-R interval. That this effect was not psychic and merely incidental to the insertion of the needle was proven by the fact that the injection of normal saline made in a similar manner on a number of occasions did not produce the slightest change in the rate. A similar but less marked early acceleration of rate was occasionally seen by Wiggers (1906). Following the increase, the rate slowed rapidly and within 1 to 2 minutes was considerably below the original resting rate. In one dog, G3, following the first fall in rate, there was a secondary rise of moderate degree and short duration, with a rapid return to the low rate. During the period of slowing, the beats tended to be grouped in twos, with an occasional single beat between. This grouping has been described previously by von Cyon (1898) and Garnier and Thaon (1906). The electrocardiographic curves resemble those of a rather marked degree of so-called sino-auricular block (fig 3). With the onset of the retardation of rate, the P-R interval increased, and the conduction defect was always exaggerated in the second of the paired beats. Within 15 to 30 minutes after the injection, the heart rate gradually rose and the P-R interval diminished. The increase in auriculo-ventricular conduction time tended to outlast the disturbance in stimulus formation (or in intra-auricular conduction, if the view is accepted that an actual block exists between the sino-auricular node and the auricle). Upon recovery, there was no sharp transition between the stages of sino-auricular block and the usual sinus arrhythmia, and the gradual merging of the one into the other suggests an intimate relationship between the two. Excepting the change in rhythm, the most striking alteration in the electrocardiogram was a marked increase in the amplitude of the T wave which occurred in all three experiments about 15 seconds after the injection of the pituitary extract. In two dogs, G1 and G2, the amplitude diminished rapidly after 30 to 45 seconds, while in the third animal the T wave became lower, although it was rather high throughout the experiment.

Table 2 gives briefly the results obtained in these experiments. In figure 4, the experiment of G1 is charted graphically.



L10 3 ELECTROCARDIOGRAM TAKEN 3 MINUTES AND 30 SECONDS AFTER THE INJECTION OF 1 CC PITUITARY EXTRACT (G3)

So called sino-auricular block is present and the conduction time is increased

TABLE 2  
*Effect of the intravenous injection of 1 cc pituitary extract on the heart before atropine*

G1			G2			G3		
Time	Rate	Conduction time	Time	Rate	Conduction Time	Time	Rate	Conduction time
Resting	115	0 13-0 15	Resting	100	0 10	Resting	124	0 12
9 seconds	171	0 13	9 seconds	134	0 10	7 seconds	160	0 12
17 seconds	85	0 14-0 16	18 seconds	90	0 09+	15 seconds	200	0 09+
1 minute and 15 seconds	47	0 13-0 15	1 minute and 30 seconds	92	0 08-0 09	30 seconds	73	0 12
2 minutes and 30 seconds	49	0 15-0 17	2 minutes and 15 seconds	48	0 10	1 minute and 20 seconds	62	0 12-0 14
6 minutes	52	0 16-0 20	2 minutes and 45 seconds	85	0 10	3 minutes	41	0 12-0 15
10 minutes	53	0 16-0 18	5 minutes and 15 seconds	46	0 11-0 12	6 minutes	57	0 12-0 16
22 minutes	52	0 16-0 20	10 minutes	52	0 10-0 13	10 minutes	57	0 12-0 17
32 minutes	60	0 16-0 18	21 minutes	68	0 11-0 12	21 minutes	77	0 14-0 17
42 minutes	58	0 15-0 16	32 minutes	77	0 10-0 11			
52 minutes	70	0 14-0 16	41 minutes	90	0 1 -0 11			
62 minutes	76	0 14-0 16						

In three experiments (table 3), small amounts of pituitary extract were injected, and in these observations records were taken during only a short time, as the effect upon the period of acceleration was sought. The results were similar to those obtained with the larger dose except that they were not so marked. In the two experiments in which only 0.1 cc was given, no rise in rate was seen. In one experiment, G2, in which 0.1 cc was administered, the electrocardio-

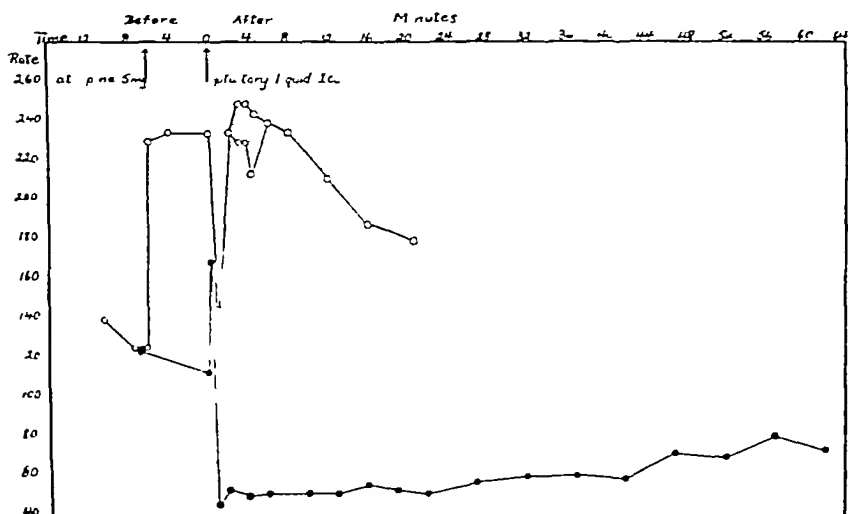


FIG 4 THE EFFECT OF THE INTRAVENOUS INJECTION OF 1 CC. PITUITARY EXTRACT BEFORE ATROPINE (LOWER CURVE) AND AFTER ATROPINE (UPPER CURVE) DOG (G1)

The shaded area in the upper curve represents auriculo-ventricular block, the top line of this area giving the auricular and the bottom line the ventricular rates. The upper curve represents one of the few instances in which the primary rise was absent.

graphic curve taken soon after the injection showed auriculo-ventricular dissociation with an auricular rate of 38 and a ventricular rate of 39, the idioventricular rhythm arising in the right bundle of the ventricular conduction system (fig 5). A similar phenomenon, i.e. the dislocation of the pacemaker to one of the bundle branches, was seen by Greene and Gilbert (1922) in one of their experiments upon the influence of anoxemia on dogs. They attributed the disturbance in the cardiac mechanism to



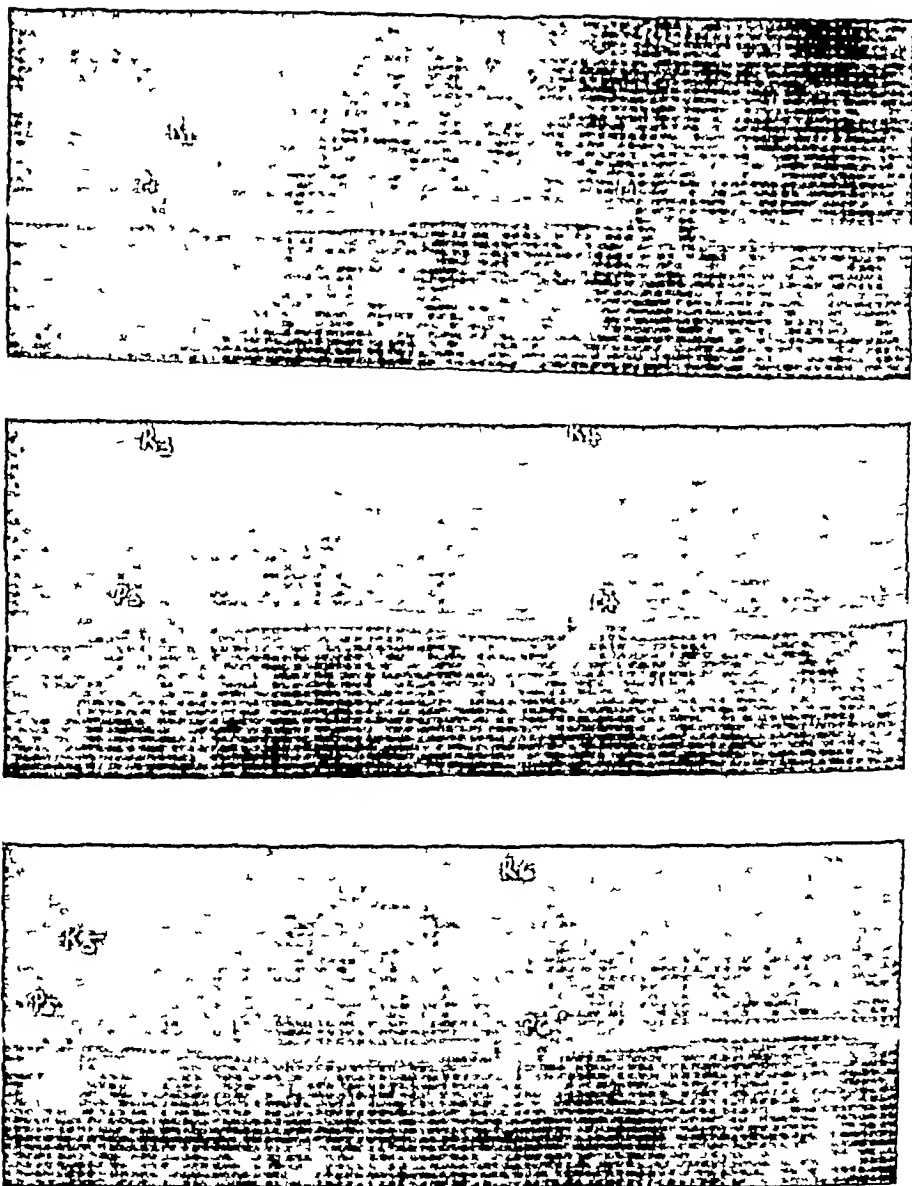


FIG 5 ELECTROCARDIOGRAMS TAKEN 1 MINUTE AND 15 SECONDS AFTER  
THE INJECTION OF 0.1 CC PITUITARY EXTRACT BEFORE  
ATROPINE (G2)

The curve demonstrates the development of auriculo-ventricular dissociation. The three curves are continuous. In the upper record, the first ventricular complex (R1) follows an auricular impulse and has the normal supraventricular form. The second ventricular complex (R2) is idioventricular in origin, arising in the right bundle branch. In the middle record, two more ventricular beats of the same type are seen (R3 and R4). In the lower record, the first ventricular complex (R5) responds to an auricular impulse and it has the normal form. The next ventricular beat (R6) however, again arises in the right bundle branch.

vagal stimulation, and this explanation probably holds in the instance here described. The striking increase in the amplitude of the T wave was absent in these experiments. In table 3 are given briefly the results of this group of observations.

*The effect of pituitary extract in animals after atropine*

In six experiments, 1 cc pituitary extract was injected within 3 to 5 minutes after the administration of atropine, which in three experiments was given in single doses of 5 mg, and in three other experiments in doses of 1 mg repeated at about 15 minute intervals (table 4). This latter method was employed in order to permit longer observations, as the animals tended to become restless after the larger amount of atropine. Very soon after the pituitary extract was given, there was a slight rise in rate which was followed rapidly by a rather marked fall of brief duration. This was succeeded by a conspicuous rise in rate which gradually and progressively fell until the close of the experiment. To facilitate the discussion of these reactions, we shall term them primary rise, primary fall, secondary rise and secondary fall respectively. In figure 4 is charted an experiment on G1. In figure 6 is shown the typical course of a longer experiment (G2).

*The primary rise.* In three of the experiments (3, 5, 6, table 4), following the injection of pituitary extract, the rate accelerated to a level above that obtained after atropine alone. The increase was slight but definite. In a fourth experiment (4, table 4), the rate had fallen rather rapidly after atropine before pituitary extract was given, and although it rose again with the administration of this drug, the level reached was below that secured with atropine alone. In two (1, 2, table 4), no change was noted. The conduction time remained the same, or increased slightly. When the rise occurred, it began 9 to 18 seconds after the injection and persisted for 8 to approximately 60 seconds.

In all these observations, a sufficient amount of time had elapsed between the injections of atropine and pituitary extract for the restoration of a certain amount of vagal tone. Three further experiments were done to eliminate any possible effect which even a slight degree

TABLE 3  
*Effect of small doses of pituitary extract on the heart after atropine*

G1			G2			G3		
0.2 cc pituitary liquid			0.1 cc pituitary liquid			0.1 cc pituitary liquid		
Time	Rate	Conduction time	Time	Rate	Conduction time	Time	Rate	Conduction time
Resting	79	0 14-0 15	Resting	76	0 09+	Resting	124	0 12+
9 seconds	110	0 13-0 14	9 seconds	84	0 09+	9 seconds	122	0 12+
14 seconds	140	0 13-0 14	14 seconds	80	0 09+	14 seconds	120	0 12+
21 seconds	68	0 14	21 seconds	74	0 10	21 seconds	73	0 12+
27 seconds	82	0 14	27 seconds	77	0 10	27 seconds	55	0 12+
2 minutes and 30 seconds	62	0 14-0 16	1 minute and 15 seconds	A-38* V-39		1 minute and 30 seconds	54	0 12-0 14

\* A-V dissociation, idioventricular rhythm arising in right bundle branch

TABLE 4

*The effect of pituitary extract after atropine\**

Number of experiment	Before pituitary extract						After pituitary extract					
	Dog testing			After atropine			Primary rise			Primary fall		
	Rate	Conduction time	Atropine mg	Rate	Conduction time		Rate	Conduction time	Time	Rate	Conduction time	Time
1	6.1	126/0	13-0	11	5	235	0 09			116/0	11	1 minute and 20 seconds
2	6.2	105	0 08	5	217/0	07-0	08			132/0	08	2 minutes
3	6.2	92	0 10	1	204	0 09	218	0 10	1 minute	156/0	10	2 minutes
4	6.2	82	0 10	1	213	0 09	230/0	10	35 seconds	110/0	09	2 minutes
5	6.3	91	0 10	5	276	0 10	258	0 10	15 seconds	222/0	15†	45 seconds
6	6.3	130/0	10-0	12	1	260	0 05	85	12 seconds	162/0	12	45 seconds

\* The rates are maximal or minimal for the particular type of reaction, and the time represents the time when these rates occurred, not the time of onset of the reaction

† In this instance the rate dropped from 213 to 206 before the injection of pituitary extract, following which the rate rose to 230

‡ The conduction time could not be accurately determined

§ Rate recorded one minute after last dose of atropine

of vagal influence might have (table 5) Six milligrams of atropine were given, and one minute later, at the height of the effect, 1 cc pituitary extract was injected. Continuous records were taken in order

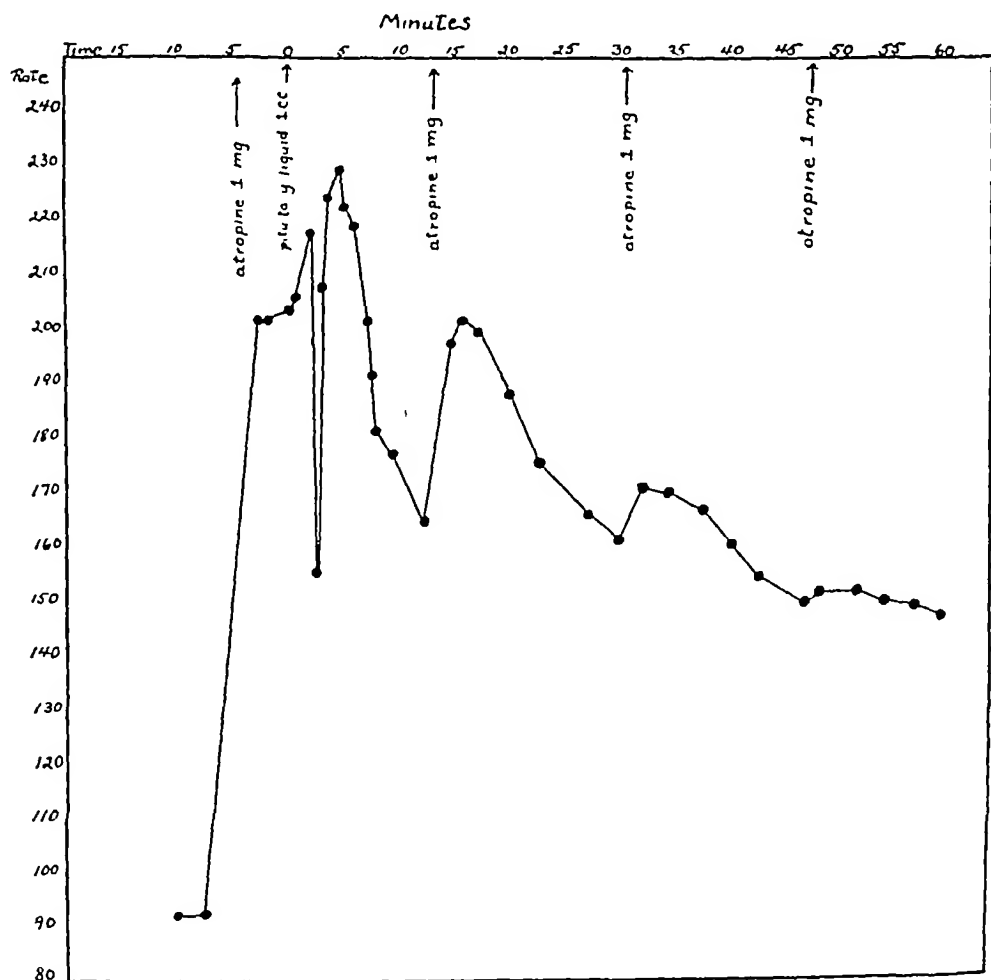


FIG 6 THE EFFECT OF THE INTRAVENOUS INJECTION OF 1 CC PITUITARY EXTRACT AFTER ATROPINE (G2)

It demonstrates the successive stages in the typical reaction the primary rise, the primary fall, the secondary rise and the secondary fall. The diminishing effect of atropine during the secondary fall is well shown

that no change be overlooked. In all three, the results were strikingly constant. Eight to nine seconds after the injection, the heart rate increased, and this acceleration lasted 9 seconds in two animals and

TABLE 5  
*Effect of pituitary extract on the heart after atropine*

	Time after pituitary extract	C1		G2		G3	
		Rate	Conduction time	Rate	Conduction time	Rate	Conduction time
Atropine 6 mg Pituitary extract 1 cc	Resting	92 240	0 14 0 10	80 258	0 09-0 10 0 08	98 280	0 10 *
	9-12 seconds	240	0 08+	262	0 07	290	*
	12-15 seconds	240	0 09	264	0 07	296	*
	15-18 seconds	234	0 09+	262	0 07	304	*
	21-24 seconds	210	0 11	260	0 08	288	*
	1 minute	165	0 13	171	0 10	200	0 13
	2 minutes	212	0 18*	110	0 10	A-285	0 19-0 22*
	2 minutes and 30 seconds					V-215 A-285	0 16-0 19*
	3 minutes	V-248 V-206 A-250 V-209	0 18-0 22* 0 18-0 22*	186 †	0 11	V-220 A-300 V-220	0 11-0 19*

\* The A-V conduction time could not be determined accurately.

† Experiment discontinued, 186 does not represent the maximum rate which might have been reached.

18 seconds in the third. The increased rate was now associated with a slight decrease in conduction time. Brief protocols of these three experiments are given in table 5.

In all experiments, there was observed the same conspicuous increase in the amplitude of the T wave seen previously in the animals before atropine, and the change took place almost constantly at the same time, about 15 seconds after the injection of pituitary (fig 7). The average duration of this alteration was about 10 seconds.

*The primary fall.* Following the short initial rise, and in most cases about 20 seconds after the pituitary extract was given, the heart rate began to fall rather rapidly, reaching a minimum in about two minutes, sometimes earlier. The lowering was considerable, varying from



FIG 7 ELECTROCARDIOGRAM TAKEN 15 SECONDS AFTER THE INJECTION OF 1 CC PITUITARY EXTRACT AFTER ATROPINE (G1)

Note the increasing amplitude of the T wave

54 to 124 beats per minute (tables 4 and 5). At this time there was a tendency for the conduction time to increase, and in some experiments the increase was marked, but in others the change was not striking.

*The secondary rise.* This took place rapidly after the lowest rate was reached, and within 1 to 2 minutes the heart rate rose to a level equal to, and usually well above the maximum following atropine alone (tables 4 and 5). This marked acceleration is the more noteworthy in that it occurred when some, and often an appreciable amount of vagal tone had been regained. In all but two of the experiments, the conduction time was distinctly increased, and in four the conduction defect was sufficient to cause the dropping out of numerous ventricular beats. Erlanger and Hirschfelder (1905) and Lewis and

Oppenheimer (1910-11) have shown that an increase in rate exaggerates depression of conduction, which in these experiments was becoming apparent in the period of primary fall

*The secondary fall* With the progress of the experiment, the heart rate gradually became slower. This change is well illustrated by figure 6 which shows the progressively diminishing effect of atropine in bringing about an increase in rate (see also table 4). With the lowering of rate the conduction time diminished, returning to normal in some cases, and remaining somewhat prolonged in others. The experiments were not continued for a sufficient length of time to determine the duration or extent of the fall in heart rate which might have taken place.

#### DISCUSSION

##### *The mode of action of pituitary extract upon the heart*

It is clear that the action of pituitary extract upon the heart is at least twofold. First, there is a direct action upon the myocardium which is shown by the primary and secondary falls in heart rate after vagus paralysis and which is substantiated by the concomitant depression of auriculo-ventricular conduction that occurred in most cases. Moreover, the progressive nature of the direct action is illustrated by the course of events in the more prolonged experiments in which the effect of atropine in causing an escape became less and less noticeable. Second, that pituitary extract acts through the vagi by a stimulation of the cardio-inhibitory center is demonstrated by the difference in the degree of slowing obtained in animals before and after atropine (fig 4). The succession of events which we have observed so constantly in the experiments upon the heart after atropine has not, so far as we know, been previously described. Wiggers (1911) believes the direct action of pituitary extract on the myocardium when it occurs, is a late effect, but our results indicate, as is shown in the primary fall and the early effect upon auriculo-ventricular conduction, that pituitary extract begins to exert a depressant action on the myocardium very soon after its administration.

The direct effect upon the heart muscle is evident, but it is difficult to determine to what extent it is a primary action, or one secondary



to constriction of the coronary arteries Although Cow (1911) and Rabe (1912) were unable to demonstrate that pituitary extract exerted a consistent effect upon the coronaries, Dale (1909), Morawitz and Zahn (1914), de Bonis and Susanna (1909) and Pal (1909) have shown that the coronaries are markedly constricted by this drug

### *The rôle of anoxemia*

The influence of pituitary extract upon the coronaries suggests that the changes in the cardiac mechanism subsequent to the administration of this drug may be, in part at least, the expression of a reaction to an insufficient supply of oxygen Kolls and Geiling (1924) have found recently that a few minutes after the intravenous injection of 1 cc pituitary extract into unanesthetized dogs (the same animals used in the present series of experiments), there occurred a striking diminution in circulatory minute volume and in oxygen consumption per minute That these effects are not merely due to a lowering of the basal metabolic rate is attested from the fact that with these changes there is seen a definite increase in the H-ion concentration of the venous blood and a lowering of the CO<sub>2</sub> combining power of the plasma of venous blood (Geiling), pointing to an accumulation of non-volatile acid (lactic) in the tissues These findings are similar to those of Meakins, Dautrebande and Fetter (1923) in cases of circulatory failure and a lowering of the circulatory minute volume Other symptoms of oxygen want appear also, such as Cheyne-Stokes respiration, and Roberts (1923) has shown in anesthetized animals that this phenomenon is due to oxygen deficiency brought about by the action of pituitary extract on the vessels supplying the medullary centers

Greene and Gilbert (1922) studied the effect of a slowly produced, progressively increasing degree of anoxemia on the heart of the anesthetized dog The rate increased up to a critical period (cessation of respiration), at which time the rate fell rapidly, an effect due to vagal stimulation Before death, however, there were evidences of a direct action upon the heart muscle Lewis and Mathison (1910-1911) investigated the effect of asphyxia in cats and found that following a short period of acceleration, the auricular rate diminished and heart

block eventually ensued. The course of events was unaffected by vagal section, and consequently was ascribed to a direct action upon the myocardium. It is probable that the discrepancy between the results is due to the difference in the methods of producing anoxemia, and that the rapidly developed, profound degree of oxygen deficiency brought about by asphyxiation caused changes in the cardiac mechanism seen only as a late manifestation of the more slowly produced anoxemia.

The results of injection of pituitary extract resemble in some respects those obtained in both the above types of experiments. The evidence of direct action on the myocardium occurred almost from the start, and it was essentially similar to that seen by Lewis and Mathison, that is depression of conduction and slowing of the auricular rate. Actually, this direct effect appeared sooner in our experiments, and this may be due to the sudden constriction of the coronaries almost immediately after the injection. As in the experiments of Greene and Gilbert, the vagal influence was most pronounced in the earlier phases of the reaction, to be followed later by a predominantly direct action on the heart muscle. Whether anoxemia played any part in enhancing vagal tone we cannot ascertain, for the elevation of blood pressure produced by pituitary extract may in itself account for this effect. In the experiments of Greene and Gilbert and of Lewis and Mathison, there was noted the same alteration of the T wave described above.

### *The primary rise*

Although the primary rise was not constantly found, it was in most instances definite. Since it occurred after full doses of atropine as well as before atropine, vagal release may be disregarded as an explanation of the quickening of rate. Usually it occurred about 8 to 10 seconds after the injection of pituitary extract was begun, which corresponds fairly well with the amount of time necessary for the drug to reach the heart (Stewart). The rise was probably due to a direct action on the muscle, rather than to stimulation of the sympathetic nerve endings which are unaffected by pituitary extract (Dale, 1909).

*The secondary rise*

This reaction usually took place at a time when a certain amount of vagal tone had been regained. Nevertheless the rate rose in most of the experiments not only to the level reached after the administration of atropine alone, but often definitely above it. There are several factors which may contribute to this acceleration and the following explanation is offered as a tentative hypothesis. The increase in systemic blood pressure reaches its height about the time when the secondary rise begins. Such an action would tend to counteract the effect of coronary constriction which is probably responsible for the primary fall. This increase in coronary flow would be further augmented by the metabolites of asphyxia (Markwalder and Starling, 1913). The increase in heart rate may be also due in part to the action of adrenal secretion which is called forth by asphyxia (Cannon, 1919)<sup>3</sup>

In only one of three animals to which 1 cc pituitary extract had been given before atropine did an elevation of rate, corresponding to the secondary rise seen after atropine, take place. The absence of this rise was probably due to the powerful vagal stimulation which was present at the same time.

## SUMMARY AND CONCLUSIONS

1 In the heart of unanesthetized dogs, pituitary extract given intravenously causes

*a* A brief period of acceleration,

*b* A profound slowing with prolongation of auriculo-ventricular conduction time. During this phase, electrocardiographic curves show so-called sino-auricular block.

2 After atropine, pituitary extract brings about

<sup>3</sup> Since the completion of this paper, our attention has been directed to a paper by Emis (Biochem, Ztscht, 1913, li, 96) who observed an elevation of rate in isolated mammalian hearts about 1 to 2 minutes after the administration of pituitary extract. This would indicate that the rise in heart rate occurring at this time may be independent of the effect of blood pressure and of the action of adrenal secretion. Since Emis' results differed however from those obtained by others using isolated hearts (Hedbom, 1908, Cleghorn, 1899, Dale, 1909, Wiggers, 1911) we must await further evidence bearing upon this question.

*a* An early period of acceleration, followed by

*b* A marked fall in rate, often associated with slight depression of auriculo-ventricular conduction This is succeeded by

*c* A conspicuous elevation of the heart rate, usually with a definite depression of auriculo-ventricular conduction Following this there occurs

*d* A progressive fall in rate, independent of the influence of vagal tone

3 From the above observations, we conclude that under the conditions of our experiments, pituitary extract affects the heart

*a* By stimulation of the vagi through the cardio-inhibitory center, and

*b* By a direct action upon the myocardium

*c* There is possibly an indirect action on the sympathetic nerve endings during the secondary rise, through stimulation of the adrenals by asphyxia

4 There are similarities between the effects of pituitary extract and anoxemia on the heart.

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# THE ACTION OF PITUITARY TARTRATE ON THE HEART OF THE UNANESTHETIZED DOG

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## INTRODUCTION

In another paper (Resnik and Geiling (1925)) we have described the effect of extracts of the posterior lobe of the pituitary (Armour's Pituitary Liquid) upon the heart of the unanesthetized dog. It was found that this substance quickly produced a period of acceleration followed by a profound slowing of the heart rate associated with depression of auriculo-ventricular conduction. During the slowing, the electrocardiographic records resembled those described as sino-auricular block. When the drug was given after a full dose of atropine however a different effect was observed. There was a brief phase of acceleration followed by a marked slowing of the heart accompanied by slight depression of conduction. A second period of acceleration followed, during which the auriculo-ventricular conduction was usually greatly impaired and the heart rate often elevated well above the maximum rate attained after complete vagal paralysis. A prolonged period of progressive diminution of cardiac rate then occurred which was independent of the influence of vagal tone. These effects were found to be due to central vagal stimulation and also to a direct action upon the myocardium. The second period of acceleration was apparently due in part to an outpouring of adrenal secretion called forth by the diminution of oxygen supply to the tissues, which occurs according to Kolls and Geiling (1924), after administration of pituitary extract.

Abel, Rouiller and Geiling (Abel, 1924) have investigated the action of pituitary tartrate, which they consider to contain the purified principle of the extract of the posterior lobe of the pituitary gland. They have found that it reproduces exactly the various effects obtained with commercial extracts. Through the kindness of Drs J J Abel and C A Rouiller, we have obtained a small amount of pituitary tartrate (oxytocic titer  $160 \times \beta$ -I phosphate) which we have used in a study of its action upon the heart of the unanesthetized dog.

## METHODS

The experiments were performed upon two of the dogs previously used in the study of the action of pituitary extract. The methods

were similar to those employed in the former experiments and need not be given in detail here Pituitary tartrate was given in doses of 1 mg

#### EXPERIMENTAL

Owing to the limited amount of the drug at our disposal we were able to perform but four experiments Nevertheless these few observations were sufficient to indicate that the purified pituitary tartrate duplicated in every important detail the action of the commercial extract.

##### *Effect upon the animal before atropine*

This was studied in two experiments (table 1) About 10 seconds after the start of the injection, the rate rose rather markedly and this rise was associated with a quickening of auriculo-ventricular conduction These changes were followed rapidly by a retardation of the rate of the whole heart, associated with a definite depression of auriculo-ventricular conduction Electrocardiographic records showed sino-auricular block during the period of slowing In table 1 are given protocols of these experiments Atropine was given after the slowing occurred with the results that are described later

##### *Effect upon the animal after atropine*

In two experiments (table 2), pituitary tartrate was given after full doses of atropine Shortly after pituitary tartrate was injected, there was an elevation of heart rate above the maximum level previously reached with the use of atropine alone In one animal, G3, this rise began 10 seconds after the injection, while in the other, G1, it occurred between 10 to 30 seconds after the injection In this animal there was a simultaneous decrease of the conduction time. There then followed a fall which reached a maximum in 1 to 2 minutes, associated in both experiments with a slight increase in auriculo-ventricular conduction time The heart rate then rose again In one instance, G1, the maximum reached was well above that obtained with atropine alone, and during this period there was heart block with the dropping of many ventricular beats In the other case,

TABLE 1  
*The effect of pituitary tartrate in animals before atropine*

G1				G3			
	Time after pituitary tartrate	Rate		Time after pituitary tartrate	Rate		Auriculo-ventricular conduction time
		Auricular	Ventricular		Auricular	Ventricular	
Resting		80	80		84	84	0 11-0 12
1 mg pituitary tartrate				Resting			
	12 seconds	107	107	1 mg pituitary tartrate	178	178	0 10
	15 seconds	55	55		74	71	0 12-0 14
	4 minutes	35	35		63	63	0 14-0 16
	10 minutes	42	42		60	60	0 14-0 17
	30 minutes	62	62				
	31 minutes			1 mg atropine	140	67*	0 13-0 16
1 mg atropine							
	32 minutes	224	224		252	252	0 16
	37 minutes	240	240		202	202	0 12
				1 mg atropine	252	252	0 12
				1 mg atropine	160	160	0 11
					250	250	0 10

\* A-V block with varying 1, 2, 1, 3, 1 ventricular response



TABLE 2  
*The effect of pituitary tartrate in animals after atropine*

G1				G3			
	Time after pituitary tartrate	Rate		Time after pituitary tartrate	Rate		Auriculo-ventricular conduction time
		Auricular	Ventricular		Auricular	Ventricular	
Resting							
1 mg atropine		104	104	Resting	100	100	0 11-0 12
1 mg pituitary tartrate		226	226	1 mg atropine	261	261	0 08
				1 mg pituitary tartrate			
	30 seconds	238	238	10 seconds	282	282	0 08
	1 minute	204	204	2 minutes	218	218	0 12
	2 minutes and 30 seconds	252	228	3 minutes	254	254	0 16
	7 minutes and 15 seconds	200	200	14 minutes and 20 seconds	224	224	0 12
1 mg atropine	9 minutes			15 minutes			
	10 minutes	242	242	17 minutes	230	230	0 13-0 14
	38 minutes	188	188	24 minutes	215	215	0 12
1 mg atropine	40 minutes			26 minutes			
	41 minutes	208	208	27 minutes	210	210	0 12
	48 minutes	192	192	31 minutes	205	205	0 11

\* The conduction time could not be measured accurately

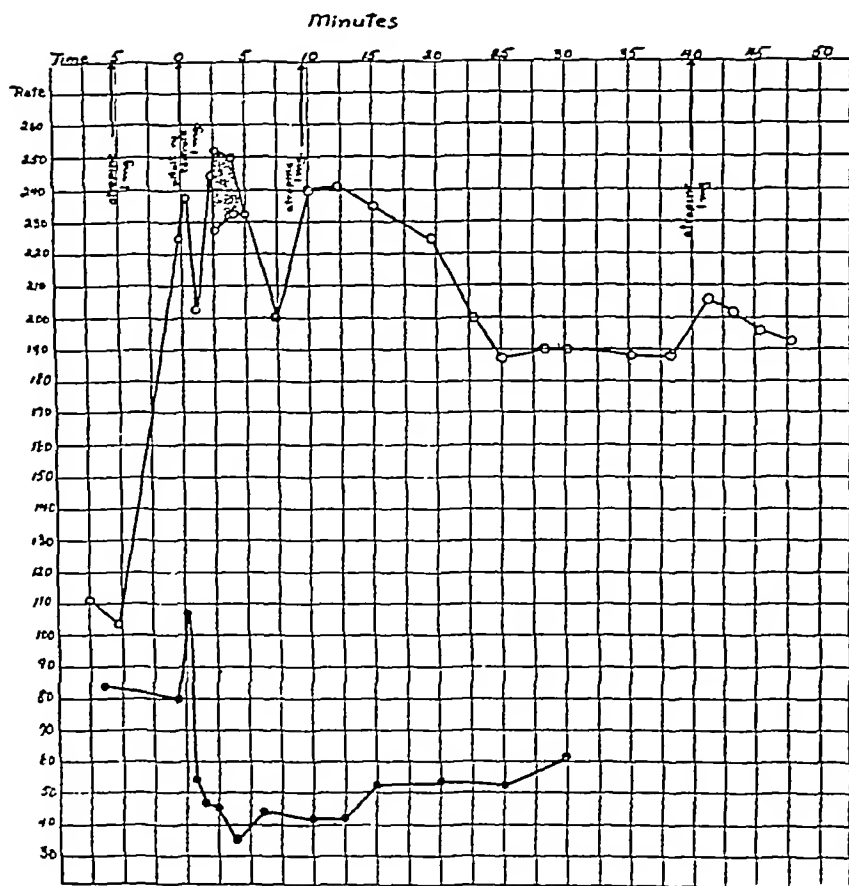


FIG 1 A chart showing the effect of the intravenous administration of 1 mgm pituitary tartrate before (lower curve) and after (upper curve) atropine (G1)

The shaded area in the upper curve represents auroculo-ventricular block and shows the difference between aurocular and ventricular rates. In the lower curve, there is seen the early acceleration and the later period of retardation of rate. In the upper curve, there is shown successively (1) the primary rise, followed by (2) a brief period of slowing, (3) a secondary rise succeeded by (4) a gradual fall during which period the effect of the third dose of atropine (40 minutes) is distinctly less than that of the second (9 minutes). Compare with figures 1 and 2 of the preceding paper.

G3, the rate rose to a level slightly below that with atropine, and the conduction time increased definitely, but no auricular beats were blocked. The heart rate gradually fell again. This fall results partly from recovery of vagal tone, but not wholly, for subsequent injections of atropine had less and less influence in effecting an increase in ventricular rate. During this period the conduction time gradually diminished. Table 2 gives the essential data of these experiments. Figure 1 illustrates graphically the results in one animal, G1.

### DISCUSSION

In those experiments in which atropine was given after pituitary tartrate, the results indicate definitely that central stimulation of the vagi was the chief factor in the slowing produced by pituitary tartrate. In G1 there was no conclusive evidence that the drug exerted a direct action on the myocardium. It is possible that such an action had been present but had passed off by the time atropine was given, although when pituitary tartrate was given to the same animal after atropine, the direct effect on the heart muscle was still evident after 41 minutes. In G3 the heart rate was slightly lower after atropine than that obtained in other experiments. More definite evidence of a direct action is seen in the increased conduction time. Following the use of atropine, the conduction time on several occasions was 0.08 to 0.10 second. In this experiment it was as high as 0.16 second. It is apparent, however, that the action of pituitary tartrate upon the heart muscle is more pronounced when the drug is given after atropine. Whether this holds for commercial preparations of pituitary extract we have not determined.

The effect of pituitary tartrate upon the heart of the unanesthetized dog is exactly similar to that of the commercial preparation which we had previously studied. The interpretation of the various phases of the effect has been given in the preceding paper. The differences between the actions of the two are merely quantitative, as was to be expected, as 1 mg. of pituitary tartrate of the particular titer which was available had been found in other experiments to be slightly less effective than 1 cc. of pituitary extract (Armour). These results offer further evidence that pituitary tartrate contains the active principle of the extract of the posterior lobe of the pituitary.

## CONCLUSIONS

Pituitary tartrate produces exactly the same changes in the heart of the unanesthetized dog that have been found to occur after the administration of pituitary extract

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# CARBOHYDRATE METABOLISM IN NEPHRITIS

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## INTRODUCTION

In an attempt to obtain further information on the incidence and significance of the hyperglycemia which has sometimes been observed in nephritis, we have studied the changes in the sugar content of the blood and urine of patients with nephritis and their respiratory metabolism following the oral administration of glucose

That the fasting level of the blood sugar was increased in nephritis was observed by Neubauer (1) in 1910. He found that there was hyperglycemia in a large proportion of cases with very high blood pressure, and he attributed both of these conditions to excessive adrenal activity. Von Noorden (2) accepted this hypothesis, but the majority of subsequent observers rejected it, emphasizing the fact that although hyperglycemia was rarely found in nephritis without hypertension yet extreme hypertension frequently occurred without hyperglycemia. Other etiological factors were sought and its association was described with uremia, edema and dyspnea (Bing and Jakobsen, (3)), urea retention (Myers and Bailey (4)), increase in blood diastase (Myers and Killian (5)), derangement of metabolism occurring late in the disease (Williams and Humphreys (6)) and with uremia, pancreatic arterio-sclerosis or hypertension with heart failure and stasis (Kahler (7)). Härtle (8) found that actual hyperglycemia was rare except in essential hypertension, but that the blood sugar was nearly always at the upper limit of normal in nephritis with increase of blood pressure. Hyperglycemia without hypertension appears to be rare, but Hopkins (9) found three instances in ten observations. The association of arterio-sclerosis, obesity and glycosuria has been recognized for many years.

The effect of glucose administration upon the blood sugar in ne-

phritis was studied by Tachau (10) and by Bing and Jakobsen (3) who from a very few observations concluded that the effect was the same as in health. Hopkins (9), Hamman and Hirschman (11) and Bailey (12) found that the blood sugar curve was definitely abnormal when there was hypertension, the rise was exaggerated and the fall delayed. Hamman and Hirschman obtained a normal curve from a patient with edema and without raised blood pressure, and also from a case of diffuse nephritis with extreme hypertension. O'Hare (13) found definite abnormalities in 11 out of 18 cases of essential hypertension.

The kidney is relatively impermeable to glucose in some stages of nephritis (Hamman and Hirschman (11), Mason (14), Williams and Humphreys (6)). Glycosuria is often absent when the blood sugar is considerably above the normal threshold, which is now accepted as from 0.16 to 0.18 per cent (11, 15, 16, 17, 18, 19).

We are not aware that any observations have been made on the respiratory metabolism after glucose administration in nephritis. The object of the present work has been in part to obtain such observations in the hope that they might shed some light on the nature of the metabolic disturbance which is associated with the tendency to hyperglycemia.

#### METHODS

The basal metabolism determinations were made by the Tissot spirometer method (20). The Henderson modification of the Haldane gas analysis apparatus was used (21). The protein metabolism was measured by determination of the urinary nitrogen by the Kjeldahl method. Protein when present was removed from the urine by heating and adding trichloroacetic acid until no further precipitate was formed, the mixture was diluted to a volume, filtered, and the filtrate used for the nitrogen determinations. The carbohydrate metabolism was calculated from the non-protein heat production and non-protein respiratory quotient by the table given by Williams, Riche and Lusk (22).

The blood sugar determinations were made by the method of Hagedorn and Jensen (23). Foster (24) has shown that after glucose administration the sugar content of the blood obtained by finger prick is greater than that of venous blood. Folin and Berglund (19) believe that this difference is chiefly due to the greater pain and psychic effect of finger pricking, but Foster (24) sees in it evidence of the burning and storing of carbohydrate in the muscles of the arm, whence comes a large proportion of the venous blood obtained by venepuncture at the usual site.

In observations in which changes of renal threshold may play a part, it is advisable to determine the blood sugar under conditions as nearly arterial as possible. Lundsgaard and Möller (25) found that the blood obtained by cutaneous incision is identical in oxygen content with arterial blood. We have therefore used cutaneous blood obtained by a clean deep prick with a sharp needle. Hagedorn's method has certain technical advantages, which are that 0.1 cc. of blood is all that is needed for the determination, and that the oxidizing agent is precipitated out in the reduced form as soon as reduction has occurred, reoxidation being thereby avoided.

The sugar content of the urine was tested qualitatively by the Benedict reagent and quantitatively by the Benedict and Osterberg (26) method. The albumin free filtrate prepared for the Kjeldahl determinations was used for the quantitative determinations.

*Glucose ingestion tests* The subjects of these studies had been used previously by us for metabolism or else were trained by blank experiments, to accustom them to the proceedings so that psychic factors might be eliminated on the day of the test. On this day, two basal metabolism determinations over five minute periods were made after 12 to 15 hours of fasting and 30 minutes complete relaxation in bed. Blood was then taken for blood sugar determination, and urine collected. The glucose was given in 180 cc. of water and 20 cc. of lemon juice at room temperature. All times were counted from this point. Blood specimens were taken at 0.5, 1, 1.5, 2, 3, and 4 hours, and urine specimens at 1, 2 and 4 hours. Metabolism determinations were made during the five minute periods preceding the 0.5, 1, 2, 3, and 4 hour points.

The dose of glucose was made proportional to the normal basal requirements estimated from the height, weight (without edema), age and sex (27). The standard amount was 100 grams for an estimated normal basal rate of 1800 calories. The glucose given varied between 1.42 and 2.57 grams per kilo body weight. In some of the blood sugar determinations without metabolism determinations the dose was 100 grams for adult males, 80 to 100 grams for adult females and 55 grams for a boy, varying from 1.3 to 1.7 grams per kilo. The glucose used was Merck's "C.P."

#### MATERIAL

Blood sugar curves were obtained on 6 normal persons and on 12 patients with nephritis, and metabolism tests were made upon 6 normals and 7 of the patients. On 2 of the patients more than one sugar curve was obtained so that 15 curves are reported.

The blood sugar curves of the normal persons are condensed in figures 1 and 2. Figure 1 shows those without alimentary glycosuria, and figure 2 those with alimentary glycosuria. Figures 3 to 7 show the curves obtained in the different forms and stages of nephritis, the classification being that of Volhard and Fahr (28). The shaded areas



TABLE 1  
*Changes in blood sugar and metabolism after glucose ingestion in six normal subjects*

Subject	Age years	Weight kilo	Glucose ingested per kilo- gram	Fasting blood sugar per cent	Highest blood sugar after glucose per cent	Glycosuria	Basal metabolic rate, devi- ation from Dubois average normal per cent	Increase in heat production after glucose per cent	Fasting R Q	Rise in R Q	Fasting carbohydrate me- tabolism per kilo per hour calories	Maximum carbohydrate metabolism per kilo per hour calories	Carbohydrate burned in 4 hours of test gm	Comment
G L	31	63.5	1.48	0.091	0.140	—	—9.3	17.65	0.80	0.10	0.25	0.58	26	Ideal type
A B	28	64.0	1.47	0.094	0.147	—	+8.5	7.44	0.74	0.06	0.11	0.47	22	Ideal type
H R	26	66.0	1.42	0.092	0.157	—	—1.8	13.89	0.73	0.10	0.08	0.37	16	Ideal type
H C	30	61.0	1.46	0.073	0.164	+	—4.4	5.66	0.80	0.12	0.28	0.56	27	Low threshold
J M	27	58.5	1.52	0.089	0.194	+	+1.7	15.80	0.79	0.10	0.28	0.70	26	"Lag" type
C A	27	62.8	1.50	0.097	0.200	+	—1.0	8.04	0.77	0.10	0.28	0.70	26	"Lag" type

represent the zone of normal blood sugar reaction and have been drawn by taking the highest lines of figures 1 and 2, and the lowest line of figure 1

The results of the metabolism tests are given with the corresponding blood and urine sugar determinations in figures 8 to 21

Tables 1 and 2 contain further facts about the tests and about the clinical condition of the patients. Protocols of the autopsy findings are appended

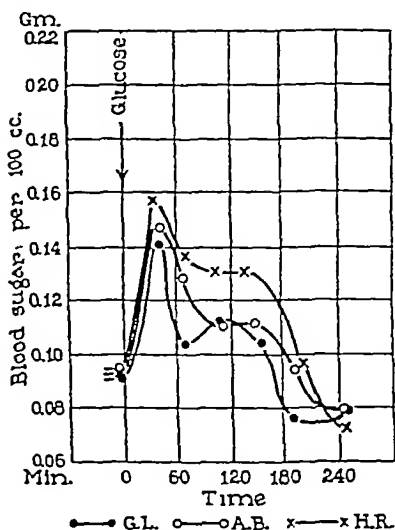


FIG 1

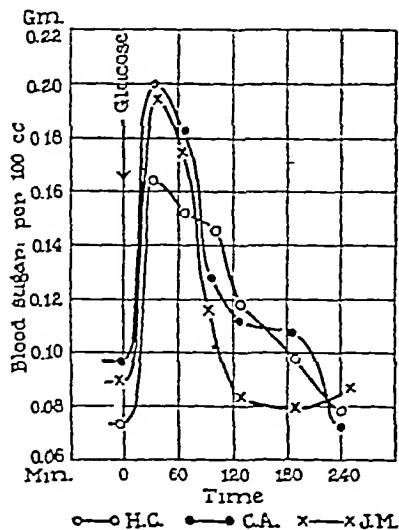


FIG 2

FIG 1 BLOOD SUGAR CURVES OF 3 HEALTHY ADULTS IN WHOM THERE WAS NO GLYCOSURIA

FIG 2 BLOOD SUGAR CURVES OF 3 HEALTHY ADULTS IN WHOM THERE WAS GLYCOSURIA AFTER TAKING GLUCOSE

The upper two curves are of the "lag" type. The glycosuria in the third case was due to a low threshold.

## ANALYSIS OF RESULTS

### *Normal blood sugar curves*

In order to obtain a series of controls for the observations on respiratory metabolism, glucose was given to six young healthy adult males

Three individuals, G L , A B , and H. R , (fig 1 and table 1), presented blood sugar curves which are normal according to recent standards, and glycosuria was absent. These ideal blood sugar curves show the following points. The fasting blood sugar is less than 0.1 per cent. After the glucose there is an abrupt rise to a peak of less than 0.18 per cent which is reached in 20 to 40 minutes. The peak is caused probably because absorption was greater at first than storage and burning. Maclean (16) suggests that this hyperglycemia is the stimulus to glycogen formation. The curve then descends steeply to a plateau 0.02 to 0.04 per cent above the fasting level and is maintained there during the second hour. During this period carbohydrate transport and storage are in equilibrium and the blood sugar comes to a constant level. When absorption and possibly the need for transport from the liver to the tissues comes to an end the curve falls to a point below the fasting level. The final hypoglycemia was observed by Frank in 1910 (29) and also by Jacobsen (15), Maclean (16), Graham (30) and Fohn and Berglund (19). It is a constant phenomenon and may appear earlier if smaller doses of glucose are given.

In the remaining normal individuals, H C , J M and C A , (fig 2 and table 1), there was sufficient sugar in the urine after the glucose to reduce the Benedict reagent. The blood sugar curves and quantitative urine sugar analyses show that two of them, J M and C A , were examples of the "lag" type of curve described by Maclean (31), tardiness in starting of an otherwise normal carbohydrate storing mechanism, and possibly unusual rapidity of absorption allowed the blood sugar to rise above 0.18 per cent for a short time. Both curves showed a rapid fall to a low plateau and a terminal hypoglycemia. The threshold appeared to be normal, and the glycosuria was apparently due to the height of the peak. The urine sugar was not increased during the fasting period or after the blood sugar had fallen (figs 12 and 13). The curve of H C did not show an abnormally high peak, but the fall was a little slow. The glycosuria was associated with a low threshold, probably at about 0.14 per cent (fig 11).

Search of the literature shows that following glucose administration, glycosuria recognizable by the ordinary methods has been found in many normal subjects. Jacobsen (15) found glycosuria in 8 out of 15 persons, Hamman and Hirschman (11) in 2 out of 6, Goto and

Kuno (32) in 33 out of 53, Hagedorn (17) frequently, and Holst (33) in 2 out of 14 healthy persons and 29 out of 145 unselected but non-diabetic patients. One of us (D D V S) when examining an army class found that 15 per cent of the members excreted small amounts of sugar after 100 grams of glucose. It is probable that some authors have not found glycosuria so often because they failed to collect a small amount of urine at the critical period, e g, Taylor and Hulton (34) conclude that "glycosuria does not occur following the largest possible ingestions of pure glucose," but they looked for it in 24 hour collections. Other authors have refused to consider as normal persons who excrete a trace of sugar after large amounts of glucose. If that were so we should have to accept the proposition that from 15 to 50 per cent of apparently healthy people are abnormal. It appears that even when allowance has been made for age (35, 36), overweight (37), worry and emotion (38, 39), there still remains a large number of healthy people who excrete sugar after consuming considerable amounts of it.

*Blood sugar determinations in patients with nephritis*

*Chronic nephrosis* Two curves on 2 patients (fig 3). The sugar curve of B B was normal except for a small rise in the fourth hour. G F was 51 years old. The curve did not rise above 0.165 but in the third hour it lay above the normal limit for young persons. In neither case was there glycosuria.

*Glomerulonephritis, nephrotic type, Stage II* Three curves on 2 patients (fig 4). Two curves were within the normal area, although that of F M after 55 grams came to its peak in the second hour. The curve of F M after 74 grams, the largest dose per kilo body weight in this series, showed a delay in falling. There was no glycosuria.

*Glomerulonephritis, nephrotic type, Stage III* Three curves on 3 patients (fig 5). All the curves lay outside the normal limits. That of S L however, was only slightly abnormal and was similar to that of F M among the Stage II curves, S L having retained a somewhat better renal function than M F or S Ly. The blood sugar of M F reached 0.245 per cent and although the descent was rapid, the curve was high throughout, the abnormality being definitely greater than a "lag" curve. The blood sugar of S Ly reached 0.227 per cent and

# CARBOHYDRATE METABOLISM IN NEPHRITIS

THE  
Observations on the blood

Nature of case	Subject	Age	Date	Body weight	Blood pressure	Edema	Blood urea nitrogen per liter	Urea excretion index* $\frac{10 D}{B \sqrt{W}}$	Urea (if abstin 24 hours)
				kilos			gm		
Chronic nephrosis	B B	23	March 4, 1924	56	110/60	+	0.081	75	6
	G F	51	November 27, 1923	81	130/80	+	0.07	50	6
Glomerulo nephritis	J O'M	16	May 27, 1924	52	138/76	-	0.155	43	17
	F M	14	February 4, 1924	39	180/130	++	0.146	26	11
			February 26, 1924	29	132/90	-	0.115	32	15
	S L.	20	December 27, 1924	65	165/120	-	0.49	12	1
	S Ly	16	March 11, 1924	42	158/100	(+)	0.68	15	In-
	M F	26	March 6, 1924	51	115/81	-	0.83	9	1
	J C.	27	November 5, 1923	60	170/117	-	0.42	14	1
			December 17, 1923	60	175/140	-	0.62	10	11
			March 28, 1924	61	195/130	-	1.75		
			April 11, 1924	53	170/110	-	2.87		
	E L.	33	December 3, 1923	57	235/100	+	0.475	10	In-
			February 7, 1924	47	220/130	-	0.379	8	
Vascular type Stage III	J L.	27	November 26, 1923	66	188/130	-	1.47	4	K
			November 30, 1923	66	185/115	-	1.54		
			December 13, 1923	64	185/105	-	1.39		
			January 9, 1924	66	192/120	-	1.46		
	A. S.	27	April 14, 1924	47	168/120	-	0.682	4.5	1
			May 1, 1924	44	160/121	-	2.24		
			May 5, 1924	46		(+)	2.36		
	H L.	30	April 3, 1924	55	170/120	-	0.080	117	17
	C T	34	November 22, 1923	74	175/105	-	0.152	33	17
	M S	26	January 28, 1924	50	196/135	-	0.097	40	17
Nephrosclerosis									

\* Austin Stillman and Van Slyke (61) D = urea output in grams per 24 hours. B = blood urea in grams per l.  
volume in l per 24 hours W = body weight in kilos Minimum normal index = 45

† Protocols of these patients were published in Jour Exper Med, 1924 xxxix, 931

‡ Three hours



showed a yet greater delay in falling. All these patients passed traces of sugar while the blood sugar was high, but the quantity of sugar eliminated was less than would be expected from a normal person with a similar rise in blood sugar (figs 18 and 19)

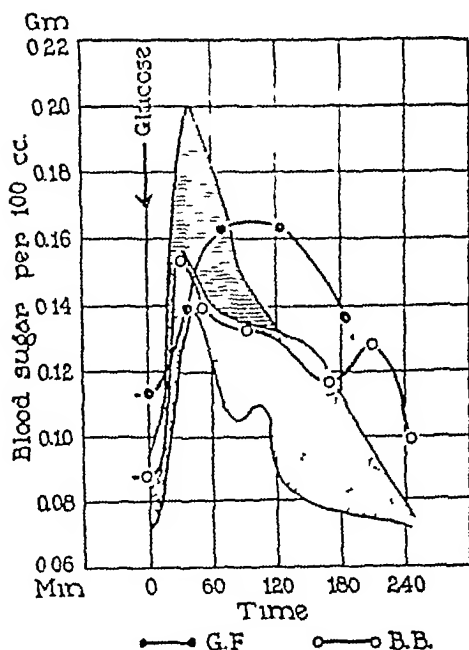


FIG 3

FIG 3 BLOOD SUGAR CURVES IN CHRONIC NEPHROSIS

There was no glycosuria. The shaded areas in figures 3 to 7 represent the normal zone delineated from figures 1 and 2

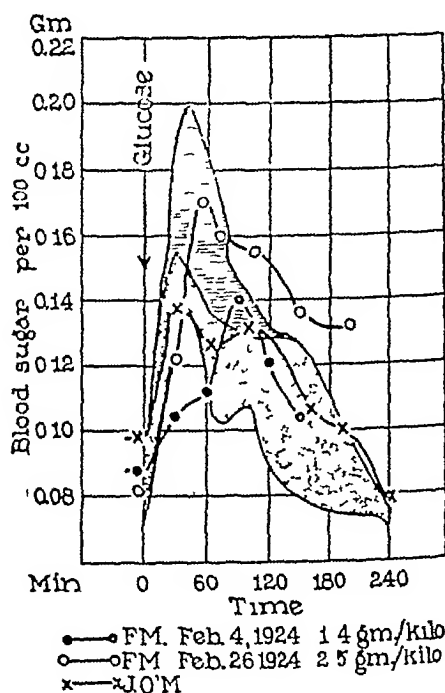


FIG 4

FIG 4 BLOOD SUGAR CURVES IN GLOMERULONEPHRITIS, NEPHROTIC TYPE, STAGE II

There was no glycosuria

*Glomerulonephritis, vascular type, Stage III* Four curves on 2 patients (fig 6). The curve of E. L. showed a high fasting level, a rise to 0.225 per cent and a steep but delayed descent. The urine habitually contained a trace of sugar which increased after the glucose was given. The threshold was low. The curves on J. C. were obtained 6 months and 5 months and 17 days before his death in uremia. The

fasting figure rose from 0.103 to 0.119 and 0.127 per cent and 3 days before death it was 0.159 per cent (table 2). None of the tests were prolonged sufficiently to show the return of the blood sugar to the fasting level, but the fall was more and more delayed and in the last

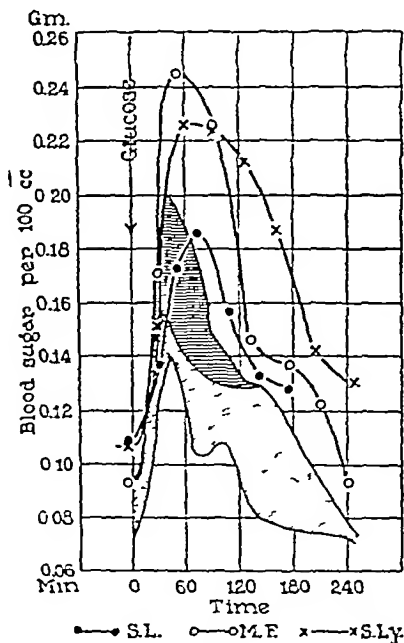


FIG 5

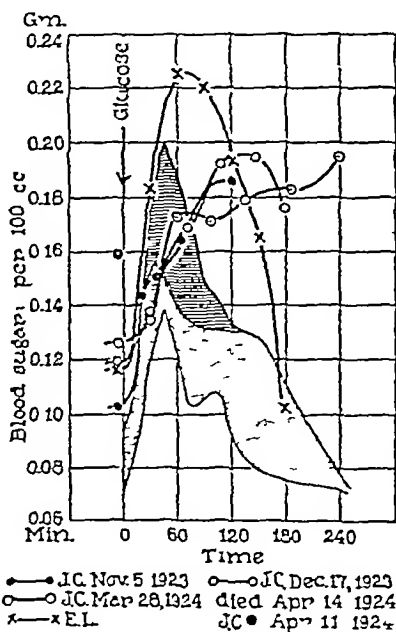


FIG 6

FIG 5 BLOOD SUGAR CURVES IN GLOMERULONEPHRITIS, NEPHROTIC TYPE, STAGE III

Glycosuria occurred in all cases

FIG 6 BLOOD SUGAR CURVES IN GLOMERULONEPHRITIS, VASCULAR TYPE, STAGE III

Glycosuria occurred in all tests except the earliest on J. C. There was a fasting glycosuria in E. L. and in J. C. in the third test

curve the blood sugar was at its highest, 0.195 per cent, at the end of the fourth hour. Glycosuria was not found in the first test and only in the third hour of the second, when the blood sugar reached its maximum, the threshold at this time being therefore in the region of 0.19 per cent. In the third test there was sugar in every specimen of urine,



including the fasting specimen, and the increased excretion after the glucose was small (fig 20). The threshold had therefore fallen to less than 0.127 per cent. From that time he constantly excreted traces of sugar.

Table 2 contains a series of determinations of the fasting blood sugar in two other patients in this stage of nephritis, A. S. and J. L. They were too sick to allow the determination of blood sugar

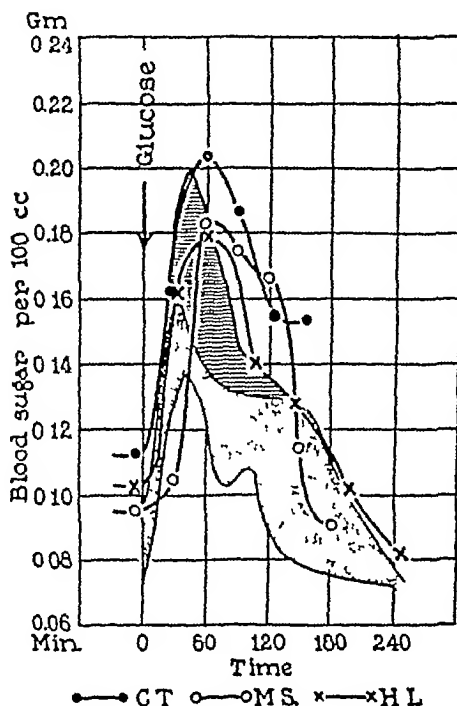


FIG 7 BLOOD SUGAR CURVES IN NEPHROSCLEROSIS, STAGE I

There was no glycosuria

curves and metabolism tests, but they showed a fasting hyperglycemia which will be discussed elsewhere.

*Nephrosclerosis, Stage I* Three curves on 3 patients (fig 7). The fasting figures were normal. The curves rose high and fell more slowly than the normal curves. They extended just beyond the normal area. There was no glycosuria so that the threshold of C. T. must have been raised to about 0.20 per cent.

In chronic nephrosis and the nephrotic type of glomerulonephritis in Stage II the blood sugar curves were substantially normal in form

and in no case was there glycosuria. In nephrosclerosis with little or no renal insufficiency the curves were all of a slightly exaggerated "lag" type, and the fasting blood sugar was towards the upper limit of normal. In both varieties of glomerulonephritis in Stage III with renal insufficiency the curves were, with one exception, S L, definitely abnormal with exaggerated rise and delayed fall, and glycosuria was frequently present. A fasting hyperglycemia was observed in the last days of life.

The abnormalities in the blood sugar curves could not be attributed in these cases to a lowered basal metabolic rate, as in the cases described by Hoxie (40). He reported that subjects with a basal metabolic rate below -10 per cent show sugar tolerance curves of the diabetic type. Although we have frequently found the basal metabolic rate lowered in nephritis, the two patients reported here with a lowered metabolic rate, B B and F M, table 2, did not present essential abnormalities in their blood sugar curves, while J C, who had a normal basal metabolic rate, presented the most extreme type of curve.

### *Carbohydrate metabolism of normal subjects*

After the glucose was taken the respiratory quotient rose 0.06 to 0.12 above the basal level, and it remained at a raised figure until the fourth hour (figs 8 to 13 and table 1). In no instance did this rise begin until the second half of the first hour. This delay after glucose is given by mouth is characteristic, and was observed by Benedict (41), Zuntz and Mering (42), Bernstein and Falta (43) and Sanger and Hun (44). Bernstein and Falta believe that the glucose is first stored as glycogen, and only when the glycogen depots are filled is it burned or converted into fat causing the respiratory quotient to rise. The type of curve obtained would thus depend upon the state of the glycogen depots, and this in turn upon the preceding diet. Higgins (45) and Bornstein and Holm (46) found that the quotient actually fell during the first 15 minutes. The latter authors suggested that this was due to  $\text{CO}_2$  being retained in the body to combine with the alkali released by secretion of acid in the gastric juice, as Bennett and Dodds (47) had found that the alveolar  $\text{CO}_2$  was increased at

that time. Such retention would result in a fall of quotient which would mask any rise due to increased carbohydrate metabolism.

A definite increase in heat production was present in half an hour and reached its maximum then in 2 cases, G L (fig 8) and C A, (figure 13), in the remaining 4 cases the maximum was delayed until one hour or later when the blood sugar curve was falling. The heat production curves of Sanger and Hun (44) were of similar form. Our curves returned to the basal level in 2 to 3 hours.

The carbohydrate combustion was less than 0.3 calories per kilo per hour during the fasting period, and it did not rise until the second half hour. The maximum combustion was attained in the second or third hour and varied from 0.37 to 0.7 calories. In the fourth hour there was a fall. In G L this fall was to 0.07 calories, and in H. R. to 0.03, in these subjects there was well marked hypoglycemia at this time, but hypoglycemia occurred in A B, H C, and J M without an excessive fall in carbohydrate combustion within the 4 hours of the test. The amount of sugar burned in the 4 hours of the test varied from 16 to 27 grams.

It appears that the accelerated metabolism of carbohydrate plethora (48) begins as soon as the blood and tissues are flooded with sugar, but that the increase in respiratory quotient occurs a little later.

Figure 14 shows the effect of 200 cc of water alone on the metabolism of the subject G L. The respiratory quotient fell and the carbohydrate combustion calculated from the gas exchange reached a low

#### *Explanation of figures 8 to 21*

The abscissae represent the time in minutes before and after the glucose was given at "zero." The ordinates have three scales. The outer scale on the left side gives the total metabolism and carbohydrate metabolism in calories per kilo per hour, and the respiratory quotient. The inner scale gives the blood sugar in grams per 100 cc. The scale on the right hand side gives the rate of sugar excretion in grams per hour.

The following signs indicate the curves

- ——— ○ blood sugar
- ——— ● carbohydrate metabolism (carbohydrate utilization)
- ——— ■ total metabolism (heat production)
- x ——— x respiratory quotient
- |——|— rate of sugar excretion

Normal  
G.L. Mar 27 1924

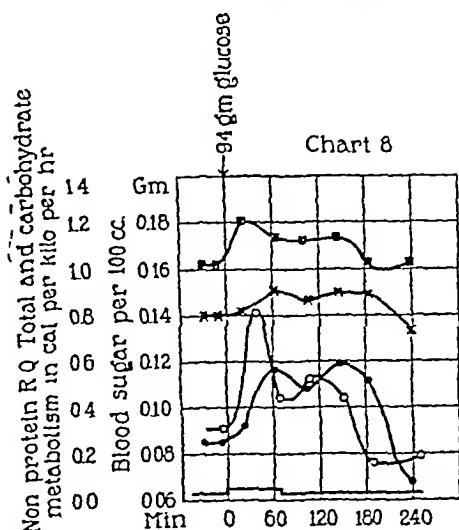


FIG 8

Normal  
A.B. Apr 1 1924

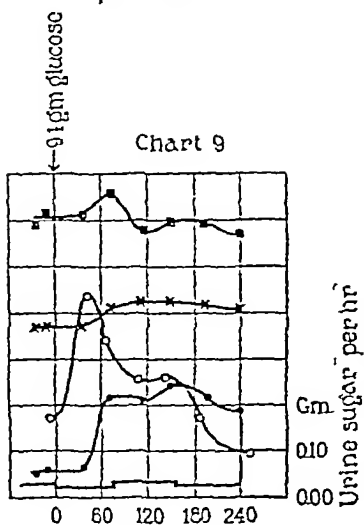


FIG 9

Normal  
H.R. June 10, 1924

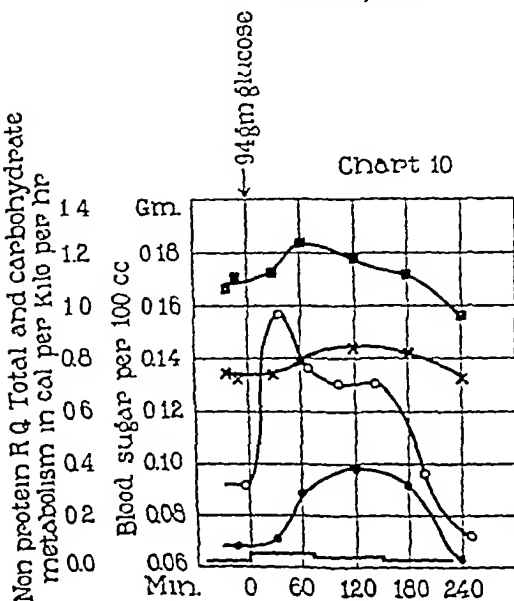


FIG 10

Normal  
H.C. June 11 1924

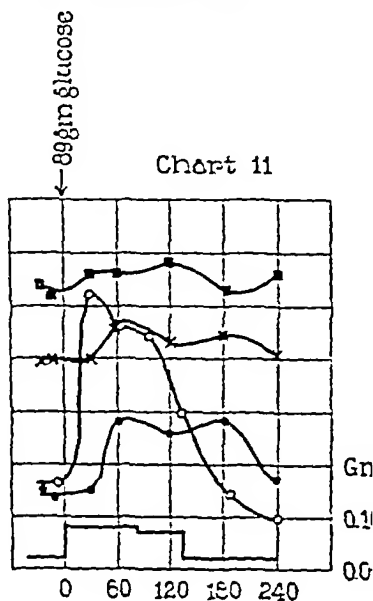


FIG 11

Non-protein R Q Total and carbohydrate  
metabolism in cal per kilo per hr

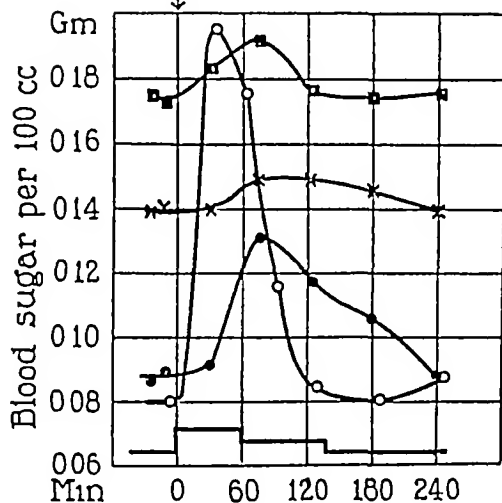


FIG 12

Control test, normal  
G L May 8 1924

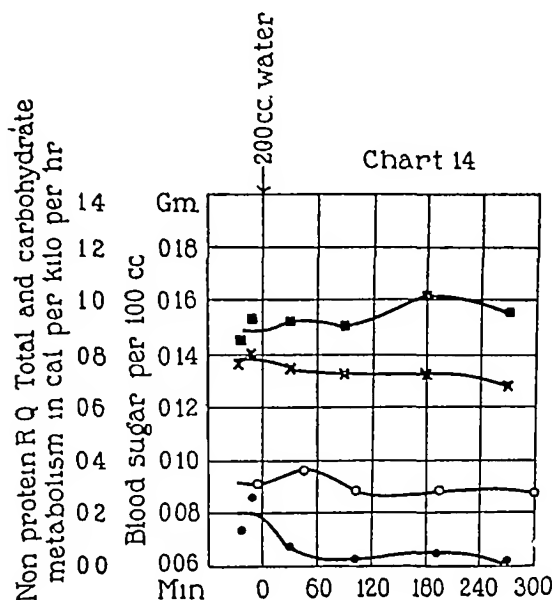


FIG 14

Normal  
C A June 12, 1924

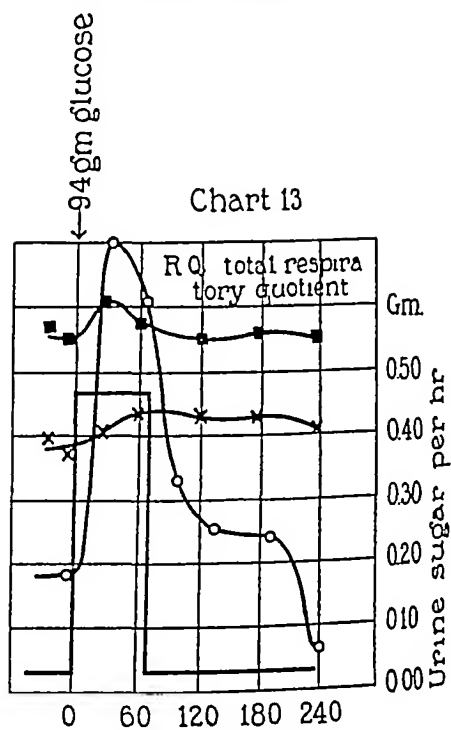


FIG 13

Chronic nephrosis  
B B Mar 4, 1924

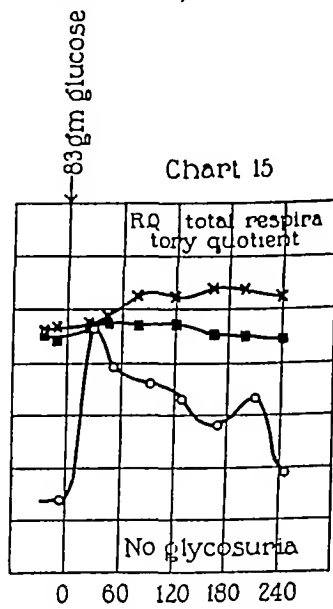


FIG 15

Glomerulonephritis nephrotic  
type stage II JOM. May 27 1924

Glomerulonephritis nephrotic  
type, stage II F M Feb. 26, 1924

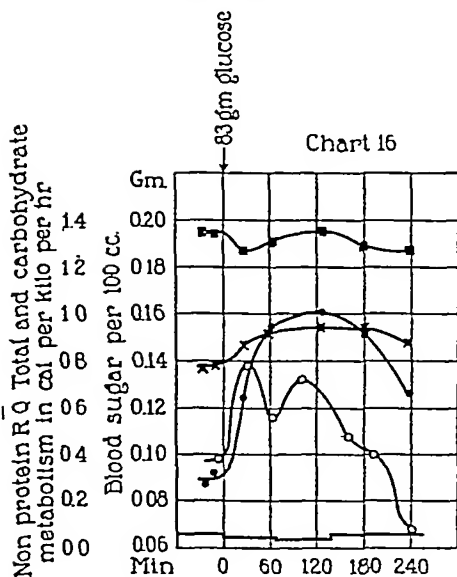


FIG 16

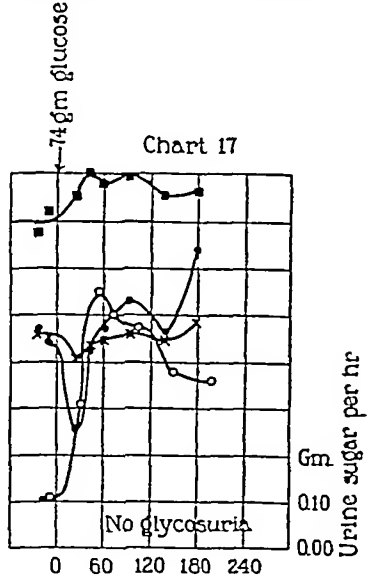


FIG 17

Glomerulonephritis nephrotic  
type stage III M F Mar 6 1924

Glomerulonephritis nephrotic  
type stage III S Ly Mar 11, 1924

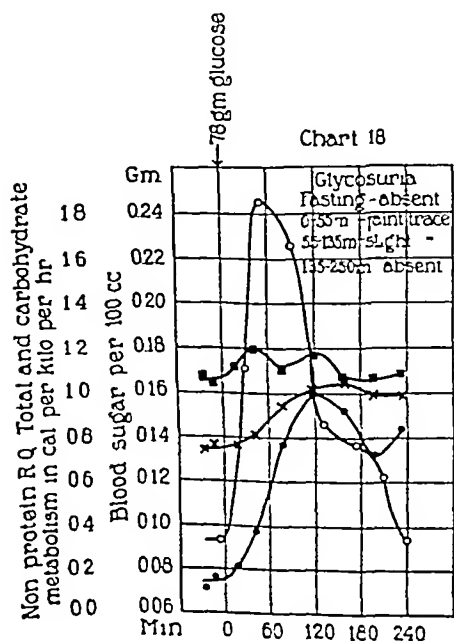


FIG. 18

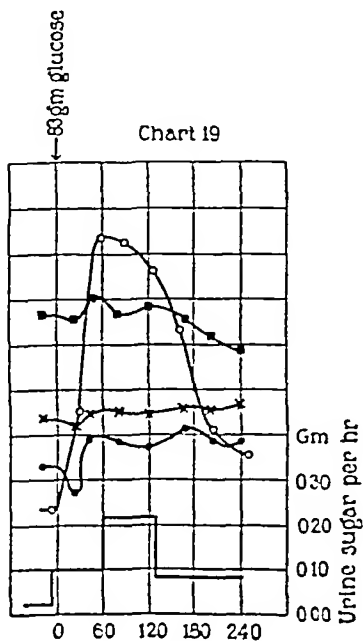


FIG 19

level. There was no significant change in the blood sugar, there was no hypoglycemia within 5 hours, that is, after 20 hours fasting. There was no effect on the basal heat production.

### *Carbohydrate metabolism of patients with nephritis*

*Chronic nephrosis* Case B B. (figure 15) *Glomerulonephritis, nephrotic type, Stage II* Cases J O'M and F M, (figs 16 and 17) B B showed a rise in respiratory quotient above unity and a normal

Glomerulonephritis vascular  
type, stage III J C Mar 28 1924

Nephrosclerosis, stage I  
H L Apr 3 1924

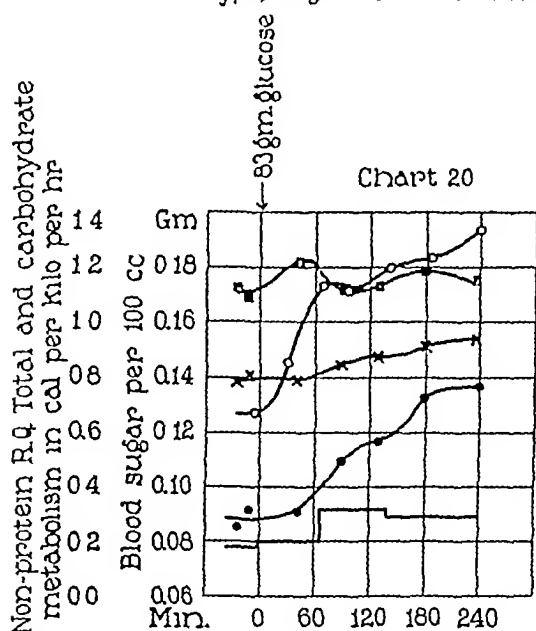


FIG 20

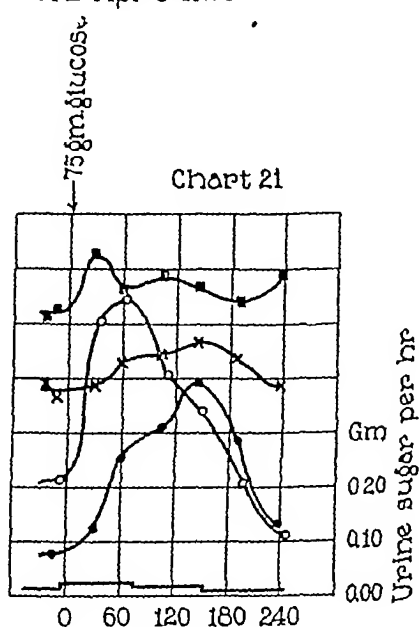


FIG 21

increase in heat production. He was being treated with urea so that his protein metabolism could not be determined, and his carbohydrate combustion could not be calculated. The height of the quotient showed that the carbohydrate was actively utilized and that some of it was converted into fat. The carbohydrate burned by J O'M before the glucose was given was 0.3 calories per kilo per hour, while immediately after the glucose it increased with a rise of respiratory quotient and reached 1 calorie. During the test he burned 40 grams of glucose. F M showed an increase in heat production which re-

maintained above the basal level until the end of the test. There was a striking transient fall of the respiratory quotient after 25 minutes and it did not rise above the initial level until the third hour. Carbohydrate combustion was 0.9 calories in the fasting state, and except for a fall at the 25 minute period it remained at a high level. The test was discontinued after 3 hours, during which time 18 grams of sugar were burned.

*Glomerulonephritis, Stage III* Cases M F, S Ly, J C (figs 18 to 20). The blood sugar curves of these patients were definitely abnormal. The heat production of M F rose immediately and returned to the basal level in the third hour. The respiratory quotient rose from 0.75 to 1.05. The carbohydrate combustion was 0.15 calories before the glucose, began to increase immediately after, and reached 0.98 calories at the end of the second hour. Thirty grams of carbohydrate were burned during the test. As in B B the rise of the respiratory quotient above unity indicated that some of the glucose was converted into fat. Both of these patients had permanent lipemia. S Ly showed but slight increases in heat production and respiratory quotient. The utilization was high in the fasting condition, 0.66 calories. It fell with the respiratory quotient at the  $\frac{1}{2}$  hour point and then rose moderately to 0.75 calories where it remained to the end of the test. Twenty-eight grams of sugar were burned. The apparent lack of response was due to the initial high level of carbohydrate metabolism. The heat production of J C increased during the first hour, but there was no change in the respiratory quotient and carbohydrate metabolism, the latter remaining at 0.3 calories. After the first hour the quotient and carbohydrate metabolism rose steadily until the end of the test, the curves running parallel with that of the blood sugar. The carbohydrate combustion reached a maximum of 0.76 calories at the end of the fourth hour, thirty grams of carbohydrate being burned in the 4 hours. The curves suggested that absorption was slow and that the increase of carbohydrate metabolism in response to carbohydrate plethora was sluggish but eventually attained normal proportions.

*Nephrosclerosis* Case H L (fig 21). All the metabolism curves were of normal form. The carbohydrate combustion rose from 0.15 to 0.78 calorie per kilo per hour and 25 grams of sugar were burned during the period of observation.



There was no constant characteristic difference between the respiratory quotients of normal persons and those of patients with nephritis after glucose administration, whether or not there were abnormal blood sugar curves. The results indicate that after glucose ingestion a somewhat more rapid carbohydrate combustion occurred in some of the nephritic patients than in the normal subjects. The differences, however, are not sufficiently marked, in view of the small number of cases, to permit a decision as to whether they are significant of the disease or are merely such variations as might be caused by previous diet and individual variation in normal subjects.

#### DISCUSSION

In the interpretation of glycosuria and supposedly pathological conditions of the blood sugar it is essential to allow for physiological variations. Spence (35) and Punschel (36) have shown that in later life alimentary hyperglycemia is prolonged, and the former has suggested that the abnormalities reported in nephritis are nothing but the normal response in the elderly. Many of the published curves, particularly those in cases of hypertension, are difficult to evaluate for this reason. Our series includes one patient of 51 years, but the remainder are under 35, so that the effect of old age has been eliminated. None of our patients were pregnant or menstruating, and none had had any recent acute infection, or any gross cerebral vascular lesion. Graham (37) found that his blood sugar curve was considerably higher when he was hard at work than just after he returned from a holiday, and this factor may apply to the normals C A and J M as they would possibly present "ideal" curves after a vacation. We have called no case abnormal unless there was a gross departure from the normal behavior of the blood sugar or carbohydrate utilization which could not be explained by some physiological condition.

Definitely abnormal blood sugar curves were obtained in Stage III of glomerulonephritis—the stage of marked nitrogen retention and isosthenuria.

The existence or not of very high blood pressure appeared to be immaterial, as S Ly and M F with blood pressures of 158/100

and 115/81 showed grossly abnormal sugar curves, although the fasting blood sugar was normal (We have not observed these nephrotic cases in the days just preceding death from uremia. In nephrosclerosis, or essential hypertension, in young persons the curves were of a slightly exaggerated "lag" type. Hypertension is not related to hyperglycemia in any constant way.

In the cases with renal edema there was no abnormality of the blood sugar.

The abnormal blood sugar curves were found in conditions of severe renal insufficiency in association with a low urea excretion index, accompanied in most cases by acidosis and phosphate retention. Cammidge (50) believes that variations in blood sugar after food are due to digestive changes in the acid-base balance of the blood, and Langfeldt (51) has demonstrated that decreasing the pH to 6.8 increases the rate of glycogenolysis by liver diastase to a maximum and advances the hypothesis that change in pH in the liver cells is one important factor in regulating the level of the blood sugar. Table 2 shows that, of the patients for whom acid-base determinations are available, all those with normal blood sugar curves had a normal acid-base balance, and all but one, E. L., of those with abnormal sugar curves had some degree of uncompensated acidosis. However, the correlation of the height of the fasting blood sugar with the degree of acid-base disturbance in the same patient is imperfect. In the case J. C. the pH and  $\text{BHCO}_3$  were more nearly normal when the fasting blood sugar was 0.159 per cent than when it was 0.127 per cent. In J. L. there was a blood sugar of 0.229 per cent and severe acidosis, and four days later after bicarbonate therapy both states were almost normal, later when the acidosis approached the initial degree the blood sugar rose to 0.139 per cent and finally with an improvement in the acidosis the sugar increased to 0.187 per cent. In the case of A. S. there was the same discrepancy. The conclusion that acidosis is the cause of the abnormal blood sugar curves does not seem justified by our data, although they are not complete enough to exclude blood reaction as a possible factor in sugar metabolism.

That phosphates take part in the carbohydrate metabolism, presumably by combining with glucose or some of the intermediate

compounds has been shown by Embden, whose work has been reviewed by Shaffer (52) and supported by the results of Harrop and Benedict (53). Renal insufficiency is likely to be accompanied by phosphate retention, as shown by Marriott and Howland (54) and by our results. If increased blood phosphate had any effect on glucose utilization, however, an acceleration rather than a retardation would be expected. Our results indicate no relationship between phosphate retention and altered carbohydrate metabolism in nephritis, except as two results of the same disease.

In the same way urea retention occurred with the abnormalities in blood sugar but no close relation was apparent.

In another communication (55) we report observations on substances in normal blood other than glucose which reduced the ordinary blood sugar reagents and find that these substances are increased in uremia. This increase, however, accounts for but a small fraction of the apparent hyperglycemia, the larger fraction being due to an increase in fermentable sugar.

The question, whether the corpuscles normally contain sugar in man or not, remains a matter of dispute. Of recent workers Falta and Richter-Quittner (56), Brinkman and Davis (57), and Csáki (58) found that human corpuscles contained no sugar, but Hagedorn (59), Ege (60) and Folin and Berglund (19) found that they contained a considerable amount. If it were assumed with some of these authors (56, 57, 58) that all the blood sugar is in the plasma, most of the high values observed in our nephritic cases could be explained as due to a relative increase in the plasma cell volume ratio. The abnormally long persistence of high blood sugar after glucose ingestion could not be so explained, and in view particularly of the analyses of Folin and Berglund (19) one cannot lay much weight on the assumption of sugar-free corpuscles.

No change in the threshold for sugar was demonstrated in the majority of the patients until the terminal stage of the disease was reached. Of the three patients with nephrosclerosis none showed glycosuria, but in one of them the blood sugar rose to 0.203 per cent and in another to 0.183 per cent, the threshold being raised in the former and probably in the latter. Towards the end of Stage III of glomerulonephritis the threshold fell to a low figure and a fasting

glycosuria appeared in three of the four fatal cases. In the fourth an adequate search was omitted. The threshold fell to the region of 0.13 per cent. The quantity of sugar excreted was small, however, even when the blood sugar rose to 0.22 and 0.24 per cent. Although the blood concentration level at which sugar escaped in the urine might be lowered, the excretion rate appeared to be retarded so that the amount lost was less than would be excreted by normal persons with an equal degree of hyperglycemia. (See figures 13, 18, 19 and 20.)

#### SUMMARY

In glomerulonephritis with severe renal insufficiency (Stage III) the blood sugar curves after glucose was given by mouth showed an exaggerated and prolonged rise, and glycosuria occurred. In the terminal stage hyperglycemia and glycosuria were present in the fasting state, the threshold for sugar being reduced.

In glomerulonephritis without severe renal insufficiency (Stage II) and in chronic nephrosis no substantial abnormality of the blood sugar was found either during fasting or after glucose feeding.

In nephrosclerosis with little or no renal insufficiency the fasting blood sugar was close to the upper limit of normal, and the rise after glucose was slightly higher and more prolonged than normal. The threshold appeared to be raised.

The rate of increase in the respiratory quotient after glucose ingestion indicated that combustion of carbohydrate was as rapid in the patients of all types as in normal subjects. The delay in the fall to normal blood sugar levels after glucose ingestion, noted in nephrosclerosis and severe glomerulonephritis, is attributable to some factor, such as retarded glycogen formation, other than failure to burn the sugar.

#### AUTOPSY REPORT

E. L. Moderate peripheral edema. Hydrothorax, about 1 liter. Heart 555 grams. Hypertrophied and dilated, no valvular insufficiency, slight coronary atheroma. Kidneys: Right 80 grams, left 70 grams. The capsule stripped with difficulty exposing a very finely granular grayish-yellow surface. On section the differentiation of cortex and medulla was obscure, and the cortex narrow. In the left kidney there was the scar of a large infarct.

*Histology* In many areas the glomeruli were reduced to hyaline scars. A few glomeruli were of large size, showed an increase of nuclei and relative bloodlessness. Most of the remainder showed epithelial crescents, hyaline and fatty changes in the tufts, adhesions and fibrous thickening of the capsule. Many tubules had disappeared, and there were fatty changes in the epithelium of the remainder. There was a great increase of the interstitial tissue and in places infiltration with small round cells. The medium vessels showed hypertrophy of the media and intima, and the smallest vessels hyperplastic intimal sclerosis.

Diffuse hyperplastic intimal sclerosis was found in the pancreas, spleen and uterus.

J. L. Fluid in both pleural and in peritoneal sacs. Heart 490 grams. Valves intact, the muscle light brown and firm. Kidneys. The left weighed 95 grams. The capsule was slightly adherent, the surface very pale and slightly granular. The differentiation of cortex and medulla was obscure. The cortical markings were indistinct and the whole cut surface appeared pale, gelatinous and homogeneous. The right kidney weighed 35 grams. There was a malformation of the pelvis, otherwise it was similar to the left.

*Histology* Large groups of glomeruli were reduced to hyaline and fibrous scars. In others there was epithelial proliferation, crescent formation and adhesions. In some the capsule was greatly thickened by concentric layers of fibrous tissue. A few were comparatively well preserved but showed hyaline degeneration of the tufts. All the glomeruli appeared bloodless. Many tubules had disappeared, and the remainder were dilated and lined with flattened or cubical cells. The arteries of medium size were greatly thickened and a few of the smallest showed hyperplastic intimal sclerosis. There was a diffuse interstitial infiltration with connective tissue and small round cells, although a few islands containing the remaining tubules and glomeruli had escaped this infiltration to some extent.

Diffuse hyperplastic sclerosis was present in the kidney and pancreas.

A. S. Weight 45 kilos. No fluid in chest or abdomen. Heart 330 grams. Mitral and aortic valves slightly thickened but not incompetent. Kidneys 90 grams each. The capsule stripped with great difficulty, leaving a pale, finely nodular surface. On section the cortex was very much reduced and the small arteries stood out prominently.

*Histology* Many glomeruli had disappeared and the remainder showed either hyaline degeneration or proliferation of the epithelial cells of the tufts and capsules, only a very few having escaped. There were numerous large "crescents." The glomeruli contained little blood. The tubules were dilated and the epithelium flattened. The coats of the medium and larger arteries were greatly thickened and the elastic lamina split into two or more layers. The smallest arteries showed hyperplastic intimal sclerosis with fatty degeneration. There was a diffuse increase of fibrous tissue in the interstitial spaces and localized areas of infiltration with small round cells.

Diffuse hyperplastic sclerosis was found in the kidneys, spleen, pancreas and liver

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# STUDIES IN SCARLET FEVER

## I STUDIES CONCERNING THE BLANCHING PHENOMENON IN SCARLET FEVER<sup>1</sup>

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### THE RELATION OF STREPTOCOCCUS HEMOLYTICUS TO SCARLET FEVER

As early as 1895, Marmorek (1) recognized the frequency with which the *Streptococcus hemolyticus* was present in the throat secretions from scarlet fever patients and inclined towards the belief that this organism might be the possible etiological factor in the production of the disease. Consequently, he prepared an antistreptococcic serum against scarlet fever by immunizing animals with polyvalent strains of *Streptococcus hemolyticus* isolated from the throat secretions of scarlet fever patients in the acute stage of the disease. Unfortunately, clinical trials with the serum boded ill for its success and it was soon forgotten. Eight years later, Tavel and Aronson (2) and Moser (3) re-examined Marmorek's work and attempted to improve the latter's method of serum production. Moser, in particular, adhered to the belief, that *Streptococcus hemolyticus* associated with scarlet fever, possessed certain specific characteristics, foreign to the streptococci usually found in other general septic conditions. This belief was not based either on cultural or on serological findings. Moser made a logical advance, however, in that he immunized a horse with strains of *Streptococcus hemolyticus*, isolated directly from the blood of fatal malignant cases of scarlet fever. He inoculated the live organisms directly into the horse, for fear that "certain specific characteristics" of these organisms might be destroyed or altered by carrying them through the usual laboratory procedures. Very promising clinical trials with this serum were reported by Moser (3), Schick (4), and a

<sup>1</sup> Read before the Medical Society of the Johns Hopkins Hospital, May 19, 1924



number of observers (5) The results were particularly impressive in the severer toxic cases, admitted to the hospital with doubtful prognosis

It was noted that ten to fifteen hours following an intramuscular injection of 200 cc of the serum, there was a rapid abatement in the toxic manifestations, a striking and critical fall of the temperature, prompt improvement in the rate and quality of the pulse, and rapid disappearance of the cyanosis with cessation of diarrhea and chills It was apparent to Schick (4), that the serum "worked essentially like an antitoxic serum and most strikingly in purely toxic cases" Difficulty arose in preserving the serum, and that combined with the frequency of serum sickness, contributed to its unenthusiastic reception and limited use

More recently attention has been focussed on the significance of *Streptococcus hemolyticus* in scarlet fever and already an enormous literature has accumulated on this subject The works of Tunnicliffe (6), Bliss (7), Stevens (8), Gordon (9), Dochez and Shermann (10), and Dochez (11), are notable in this respect They have observed that *Streptococcus beta hemolyticus*, isolated from the throats of scarlet fever patients, is a specific type of streptococcus, most readily distinguishable by agglutination and the phenomenon of agglutinin absorption, from the types of *Streptococcus hemolyticus* giving rise to angina and septic conditions in general Dick and Dick (12) working on this theory that a certain strain of *Streptococcus hemolyticus* was the specific cause of scarlet fever, swabbed a strain of this organism, isolated from a case of scarlet fever, on the tonsils of a human volunteer This resulted in what they interpreted as a mild attack of scarlet fever

Dochez (11) has recently succeeded in producing a scarlatinal anti-streptococcic serum by immunizing a horse with the specific type of *Streptococcus beta hemolyticus* found in the throats of scarlet fever patients, and has observed that this serum is capable of blanching the scarlet fever rash locally, in a more conspicuous manner than the serum of patients convalescing from scarlet fever The results obtained from its use by Blake, Trask and Lynch (13), indicate that the serum may possess distinct diagnostic and therapeutic value

Through the kindness of Dr Dochez it has been possible to use the

specific immune serum, which he has sent us, to study the blanching phenomenon in scarlet fever. During these investigations, it has seemed desirable to re-examine the ability of (a) normal human serum and (b) convalescent scarlet fever serum to blanch the scarlet fever rash, (c) to determine the exact time at which the serum from patients convalescing from scarlet fever developed the power to blanch the scarlet fever rash and (d) to compare these results with the rash extinction phenomenon obtained with Dochez's serum.

#### THE SCHULTZ-CHARLTON PHENOMENON

Schultz and Charlton (14) in 1918 first observed that intracutaneous injections of 0.5 to 1 cc. of serum from normal persons or from persons convalescent from scarlet fever caused a definite blanching of the rash in patients with scarlet fever. The blanching phenomenon occurred within five to six hours following the serum injection and the areas blanched measured from 2.5 to 5 cm in diameter. They found in a series of fifty typical cases of scarlet fever, that positive blanching reactions were obtained in forty-four, or 88 per cent, that doubtful reactions occurred in five, or 10 per cent and that there was no blanching in one or 2 per cent. The areas blanched were found to persist until the general exanthem had faded. A few years later, Neumann (15) confirmed these observations, and noted that serum withdrawn from patients during the first four days of an attack of scarlet fever, did not produce the blanching phenomenon. Hainiss (16) found in a series of nineteen cases tested with convalescent serum, that positive blanching reactions were obtained in eleven, or 58 per cent. At this time, Paschen (17) reported the results of a series of seventy-one cases tested with convalescent serum, in which positive blanching reactions were obtained in sixty-two, or 87 per cent.

The following year, Schultz (18) re-examined his earlier work and likewise confirmed the findings of Neumann. He had observed positive blanching reactions with convalescent serum in 100 per cent of scarlet fever patients tested on the second day of the disease, in 78 per cent tested on the third day of the disease and in 60 per cent of five cases each, tested on the first and the fourth day of the disease. Cases tested on the fifth day of the disease gave uniformly negative results. At this time, Tron (19) reported the results of fifty cases of scarlet fever tested with convalescent serum in various stages of the disease and observed the blanching phenomenon in only ten, or 21 per cent.

Hazelhorst (20) in 1921, obtained in a series of fifty definite cases of scarlet fever the Schultz-Charlton phenomenon in 80 per cent and reported that the tests were consistently negative in thirteen cases of other eruptive diseases. At this time, Steinkopf (21) found in a series of forty-nine cases of undoubted scarlet fever that positive blanching reactions occurred in 83.7 per cent. Furthermore,

Mulsow (22) reported a series of thirty cases of scarlet fever tested intradermally with convalescent serum withdrawn from scarlet fever patients from twenty-one to twenty-eight days after the appearance of the rash, he obtained positive blanching reactions in 53 per cent and doubtful reactions in 10 per cent. In a second series of twenty cases tested intradermally with normal human serum, positive blanching reactions were obtained in 40 per cent and doubtful reactions in 15 per cent. He also noted that serum from normal persons failed to produce the Schultz-Charlton phenomenon in about 60 per cent of definite cases of scarlet fever.

Rojo (23) in 1922 tested eighteen cases of scarlet fever with convalescent serum and obtained positive blanching reactions in 78 per cent. Raymond (24) found in a series of seventy-two cases of definite scarlet fever that intradermal injections of normal human serum produced the blanching phenomenon in 82 per cent. Meyer-Estorf (25) made the observation that the Schultz-Charlton phenomenon was best elicited on the second day after the appearance of the rash. Toomey and Nourse (26) tested fourteen cases of scarlet fever with normal human serum, using 0.5 cc doses, and obtained positive blanching reactions in nine cases, or 64 per cent. The same number of cases were tested with convalescent serum; using 0.5 cc doses, and obtained positive blanching reactions in only three cases, or 21 per cent. In a second series of twenty-seven cases, using 1 cc doses, with normal human serum they obtained positive blanching reactions in twenty-one cases, or 81 per cent and with convalescent serum they obtained positive blanching reactions in seventeen cases, or 63 per cent. Attention was called to the fact that a higher percentage of positive results can be obtained with the larger doses of serum. Blum (27) tested sixty-four cases of scarlet fever with varying amounts of serum, in an effort to show that the extent of the rash extinction was not dependent on the amount injected. The results of this work were inconstant and subject to great variations. Table 4 shows the results of the Schultz-Charlton phenomenon by other workers, together with a summary of the results of the work reported in this paper.

In reviewing the literature that has accumulated concerning the Schultz-Charlton phenomenon one finds a difference of opinion as regards the value of this test. On the one hand Neuman is inclined to regard it as a specific diagnostic phenomenon while on the other Tron considers it worthless in the diagnosis of doubtful cases of scarlet fever. In a series of closely allied investigation in scarlet fever, it seemed desirable to re-examine the blanching phenomenon with normal human serum and convalescent scarlet fever serum in order that these studies might be compared with the results obtained with the Dochez scarlatinal antistreptococcic serum.

*The blanching phenomenon with normal human serum*

In order to test the ability of normal human serum to produce the Schultz-Charlton phenomenon, blood was withdrawn from six healthy individuals, whose past histories revealed no attack of scarlet fever or other general septic conditions caused by *Streptococcus hemolyticus*, and whose Wassermann reactions were negative. The blood was placed in the ice-box for 12 to 18 hours, the serum then drawn off, heated for an hour at 56°C and stored in the ice-box in sealed tubes. To the tubes 0.3 per cent solution of tricresol was added.

Nine cases of scarlet fever were tested intradermally with doses of 1 and 0.5 cc of this serum during the first four days after the appearance of the rash. The results of these tests are shown in table 1. Of the total nine cases, five patients showed definite blanching of the rash at the site of injection on the second and third day of the disease, while two patients showed a slight or suspicious blanching reaction when tested on the second day of the disease. The greatest incidence of rash extinctions occurred on the second day of the rash, and of six patients tested on this day, four showed definite blanching. Seven patients were tested on the third day of the rash, in one only did a positive blanching reaction occur, though in one other patient a suspicious rash extinction was noted. The results were consistently negative when the tests were made on the fourth day of the rash.

The areas blanched were usually regular in outline, round or oval in shape, measuring from 1 to 6.5 cm in diameter. Some, however, were less sharply delimited, having irregular and indefinite edges with numerous stellate projections, varying in size from 5 to 9 cm in diameter. The shortest interval between the injection of serum and the occurrence of definite blanching at the site of injection was five hours, and the longest interval fourteen hours. The blanching reaction usually persisted until the disappearance of the general rash. In the areas blanched on the second day of the rash, no pigmentation or desquamation was observed, while on the third day of the rash yellow-brownish pigmentation persisted in the skin and disappeared with the general desquamation. It was found that the area blanched was directly dependent on the amount of serum injected and that while 1 cc of serum produced a definite blanching reaction, 0.5 cc. failed to extinguish the rash in the same patient. Nine control tests with horse serum and diphtheria anti-toxin gave consistently negative results. Injections of epinephrin in solutions of 1:1,000 and 1:5,000 did not produce the blanching phenomenon.

TABLE 1

*Rash extinction results from intradermal injections of normal human serum*

CASE NUMBER	DAY OF RASH	DOSE OF SERUM INJECTED	RESULTS	BLANCHING TIME	AREA BLANCHED	APPEARANCE OF AREA
		cc		hours	cm	
1	3	0.5	+	6	1.6	Regular, round
		1.0	+	5	2.4	Regular, round
	4	0.5	0			
		1.0	0			
2	2	0.5	+	9	1.2	Regular, round
		1.0	+	9	3.6	Regular, round
	3	0.5	0			
		1.0	0			
3	2	0.5	0	11	4.2	Irregular, stellate
		1.0	?			
	3	0.5	0			
		1.0				
4	4	0.5	0			
		1.0	0			
5	4	0.5	0			
		1.0	0			
6	2	0.5	+	8	1.0	Regular, round
		1.0	+	6	2.5	Regular, round
	3	0.5	0	11	6.5	Irregular, stellate
		1.0	?			
7	2	0.5	0	12	2.4	Regular, oval
		1.0	+			
	3	0.5	0			
		1.0	0			
8	2	0.5	+	8	1.5	Regular, round
		1.0	+	8	5.1	Regular, round
	3	0.5	0			
		1.0	0			
9	2	0.5	0	14	2.4	Regular, round
		1.0	?			
	3	0.5				
		1.0				

Total cases, 9, positive blanching in five cases, or 56 per cent, suspicious blanching in two cases, or 23 per cent

*The blanching phenomenon with convalescent scarlet fever serum*

In order to compare the blanching power of normal human serum with that of convalescent scarlet fever serum blood was withdrawn from scarlet fever patients twenty to forty days after the initial appearance of the rash. The convalescent serum was prepared in the same way as the normal human serum, pooled and the mixture of convalescent sera used for the following tests, the results of which are shown in table 3. A total of sixty-five intradermal injections were made in forty-two cases of scarlet fever during the first four days of the rash. The amount of serum injected varied from 0.1 to 1 cc. In a series of nineteen patients, it was found that when convalescent serum was injected intradermally during the first fifty hours after the initial appearance of the rash, the blanching phenomenon was positive in seventeen cases, or 89 per cent, and slight or suspicious after rubbing the injected area, in one case, or 5 per cent. In eight cases the injections were made sixty hours after the initial appearance of the rash. The blanching phenomenon was positive in seven of these, or 87 per cent and slight or suspicious after rubbing the injected area, in one or 13 per cent. In twenty-eight cases intradermal injection of convalescent serum seventy hours after the initial appearance of the rash, did not produce any effect upon the rash.

The blanched area in the positive cases varied from 1 to 4 cm. in diameter, the size depending directly on the amount of the convalescent serum injected into the skin. In numerous instances the blanching was just perceptible and the outline indefinite, in others the blanched area was sharply delimited, round or oval and stood out in sharp contrast to the surrounding erythematous skin. While 1 cc. of convalescent serum produced a definite blanching reaction, 0.5 and 0.1 cc. failed to extinguish the rash in the same individual and therefore the percentage of positive blanching reactions was greater with the larger doses than with the smaller amounts of serum injected. This was also observed in the reactions obtained with normal serum.

After the blanching had persisted forty to sixty hours, there appeared, as a rule, a yellowish-brown pigmentation about the site of injection, which was shortly followed by a fine, powdery-like desquamation. This peeling differed from the general desquamation which was scaly, the skin peeling off in large flakes. In most instances the blanching occurred between seven and twelve hours after the injection of the serum and persisted until the disappearance of the general rash.

Fifty control tests were made with 0.1 to 1 cc of diphtheria antitoxin or normal horse serum. These sera were injected intradermally in forty-one cases from the first to the fourth day of the rash. In no instance was the rash blanched by these control sera. Convalescent scarlet fever serum failed to produce the blanching phenomenon in five cases of erysipelas, three cases of measles and four cases of rubella.

*Initial appearance of antitoxic substance in the blood serum of scarlet fever patients.* In an effort to determine the exact time at which

TABLE 2

*Results of tests to determine the time at which serum of patients convalescing from scarlet fever attains the power of blanching the exanthem*

CASE NUMBER	AGE	DAY OF RASH	DOSE OF SERUM	SERIES OF CONVALESCENT SCARLET FEVER SERA DURING THE FIRST TWELVE DAYS OF THE DISEASE, INJECTED IN TEN CASES OF SCARLET FEVER												TIME OF BLANCHING, AVER. AGE
				1st day of serum	2nd day of serum	3rd day of serum	4th day of serum	5th day of serum	6th day of serum	7th day of serum	8th day of serum	9th day of serum	10th day of serum	11th day of serum	12th day of serum	
			cc													hours
1	3	2	0.5	0	0	0	0	0	0	0	+	+	+		+	11
2	13	2	0.5	0	0	0	0	0	0	0	0	+	+	+	+	9
3	20	2	0.5	0	0	0	?	0	0	0	0	0	0	+	+	9
4	5	2	0.5	0	0	0	0	0	0	0	0	0	+	+	+	7
5	6	2	0.5	0	0	0	0	0	0	0	0	0	+	+	+	10
6	4	2	0.5	0	0	0	0	0	0	0	0	+	+		+	12
7	8	2	0.5	0	0	0	0	0	0	0	0	0	+	+	+	7
8	8	2	0.5							0	0	0	0	+	+	10
9	8	2	0.5							0	0	0	+	+	+	7
10	11	2	0.5							0	0	0	+	+	+	8

Total cases, 10, power to produce the blanching phenomenon first observed in the eighth day serum in one case, or 10 per cent, in the ninth day serum in three cases, or 30 per cent, in the tenth day serum in eight cases, or 80 per cent, in the eleventh and twelfth day sera in all cases, or 100 per cent.

\* Suspicious blanching, followed by pigmentation and fine desquamation.

the blood serum from patients with scarlet fever develop the capacity to blanch the rash in scarlet fever, blood was withdrawn daily from a series of scarlet fever patients from the second to the twelfth day of the disease. The serum was prepared in the same manner as that described in the experiments with normal human serum. The results of these tests are shown in table 2. In a series of ten cases of scarlet fever tested with intradermal injections of the above-mentioned sera,

the first definite rash extinction reaction occurred with the serum withdrawn on the eighth day after the appearance of the rash. This occurred in only one case. The serum withdrawn on the ninth day of the disease caused definite blanching reactions in three cases and the serum obtained on the tenth day of the disease produced the blanching phenomenon in eight cases. The serum withdrawn on the eleventh and twelfth days produced definite blanching in all of ten cases. A slight or suspicious blanching reaction occurred on the fourth day of the disease, but this instance was consequently ruled out, since no blanching occurred in the same patient with serum withdrawn between the fifth and tenth days. The areas of rash extinction about the site of injection measured about 2.5 cm. in diameter and resembled in general those obtained with convalescent serum. The areas blanched by the sera removed on the eighth, ninth and tenth days of the disease were inconstant and frequently faded twenty-four hours after the initial appearance of the blanching, while the areas blanched by the sera obtained on the eleventh and twelfth days were much more striking and persisted until the disappearance of the general rash.

*The blanching phenomenon with Dochez's scarlatinal antistreptococcic serum*

During the past nine months, ninety-four intradermal tests have been made with Dochez's scarlatinal antistreptococcic serum in a series of fifty-seven cases of scarlet fever. These patients were tested during the first four days of the rash. The results are tabulated in table 3. The amount of serum injected varied from 0.1 to 1 cc. Forty cases of scarlet fever were tested within the first sixty hours after the appearance of the rash. Dochez's serum produced the Schultz-Charlton rash extinction phenomenon in all of these, or 100 per cent. Ten cases were tested seventy hours after the appearance of the rash, in only one of these, or in 10 per cent did the definite blanching phenomenon, occur, while in three or 30 per cent suspicious blanching was observed. When the test was performed eighty hours after the appearance of the exanthem, blanching, with one possible exception, was never observed.

Case no. 6 (table 3) afforded a striking and instructive example of



TABLE 3

*Rash extinction results from intradermal injections of Dochez's scarlatinal antistreptococcal serum and convalescent scarlet fever serum in early cases of scarlet fever*

CASE NUMBER	AGE	DAY OF RASH	APPROXIMATE HOURS	DOSE	RESULTS				
					Dochez	Convalescent	Control	Blanching time	Diameter of blanching (Dochez's)
				cc.				hours	cm
1	15	2	40	0.5	+	+	0	9	6.0
2	11	3	70	0.5	0	0	0		
3	33	3	70	0.5	0	0	0		
4	3	2	46	0.5	+	+	0	11	8.5
5	21	3	70	0.5	0	0	0		
6	9	2	35	0.5	+	+	0	9	7.5
				0.2	+	+	0	9	4.8
		3	60	0.5	+	+	0	12	5.0
				0.2	+	0	0	14	3.5
				0.5	+	0	0	16	4.0
				0.2	+	0	0	17	2.4
		4	80	0.5	0	0	0		
				0.2	0	0			
7	13	2	40	0.5	+	+	0	14	7.0
8	4	4	80	1.0	0	0	0		
				0.5	0	0			
9	22	3	70	0.5	?	0	0	7	8.0
10	20	2	45	0.5	+	+	0	8	4.0
11	10	3	70	1.0	0	0	0		
				0.5	0	0			
12	6	3	70	1.0	?	0	0	11	9.8
				0.5	0	0			
13	9	3	70	1.0	0	0	0		
				0.5	0	0			
14	6	3	70	1.0	0	0	0		
				0.5	0	0			
15	5	2	40	0.5	+	+	0	16	4.6
16	6	3	60	0.5	+	?	0	15	9.0
17	6	2	35	0.5	+	+	0	12	4.8
18	8	3	55	1.0	+	+	0	8	9.2
				0.5	+	+	0	9	4.6
19	4	2	40	0.5	+	+	0	14	6.8
20	8	2	45	0.5	+	+	0	17	4.4
21	2	2	40	0.5	+	+	0	16	11.2
22	12	2	30	0.5	+	+	0	12	7.5
				0.2	+	?		12	4.2
				0.1	+	0		12	2.6
23	9	2	40	0.5	+	+	0	9	9.1

TABLE 3—Continued

CASE NUMBER	AGE	DAY OF RASH	APPROXIMATE HOURS	DOSE	RESULTS				
					Dochez	Conva- lescent	Control	Blanching time	Diameter of blanching (Dochez's)
				cc.				hours	cm.
24	9	2	45	0.5	+	?	0	11	7.0
{ 25	15	3	60	1.0	+	+	0	12	7.3
				0.5	+	0		12	3.2
26	3	4	90	1.0	0	0	0		
				0.5	0	0			
{ 27	4	4	85	1.0	0	0	0		
				0.5	0	0	0		
28	20	3	60	0.5	+	+	0	12	9.0
{ 29	8	4	90	1.0	0	0	0		
				0.5	0	0	0		
30	13	3	52	0.5	+	+	0	13	5.4
{ 31	33	2	30	0.5	+	+	0	14	7.2
				0.2	+	+	0	14	3.8
				0.1	+	0	0	14	2.3
{ 32	3	1	22	0.5	+	+	0	16	3.2
				0.2	+			16	1.7
				0.1	+			16	1.3
{ 33	24	3	50	0.4	+		0	18	5.0
				0.2	+			18	4.2
34	8	2	34	0.4	+		0	9	4.0
35	13	3	55	0.4	+	+	0	11	7.6
36	8	2	36	0.3	+	0	0	7	5.4
37	30	2	40	0.4	+		0	9	4.0
38	11	4	80	0.5	?	0	0	16	6.4
39	7	2	38	0.4	+		0	7	7.2
{ 40	4	5	100	1.0	0				
				0.5	0				
41	3	3	56	0.5	+			8	7.0
{ 42	10	2	42	0.5	+			7	9.2
				0.2	+			7	4.0
				0.1	+			7	2.3
{ 43	5	5	100	1.0	0				
				0.5	0				
{ 44	12	2	38	0.5	+			9	9.0
				0.1	+			9	2.4
{ 45	11	3	50	1.0	+	+		11	8.3
				0.5	+	+		11	5.2
{ 46	3	3	55	0.5	+	+	0	14	7.4
				0.1	+	0		14	1.6

TABLE 3—*Concluded*

CASE NUMBER	AGE	DAY OF RASH	APPROXIMATE HOURS	DOSE	RESULTS				
					Dochez	Convalescent	Control	Blanching time	Diameter of blanching (Dochez's)
				cc				hours	cm.
{ 47	13	1	14	0.5	+			6	6.1
				0.2	+			6	3.3
				0.1	+			9	1.6
{ 48	15	3	56	1.0	+			12	5.6
				0.5	+			12	2.0
{ 49	6	3	60	0.5	+			13	3.2
				0.2	+			13	1.4
{ 50	7	3	55	1.0	+			9	3.0
				0.2	0				
51	11	4	90	1.0	0	0			
52	6	4	90	1.0	0	0			
53	8	2	30	0.5	+	+		9	4.7
54	6	2	40	0.5	+	+		11	6.4
55	23	2	46	0.5	+			7	4.9
56	10	2	35	0.3	+			9	7.1
{ 57	39	3	70	1.0	0*	0			
				0.5	0				

\* Suggestive blanching after rubbing the area injected

the blanching phenomenon, following intradermal injections of equivalent doses of convalescent serum and Dochez's serum, during the second, third and fourth day of the disease. A comparative study of twenty intradermal injections was made in this patient. When doses of 0.5 and 0.2 cc of convalescent serum and Dochez's serum were injected intradermally about 35 hours after the onset of the rash, positive blanching reactions were obtained within nine hours following the injections, the size of the areas blanched varying directly with the amount of serum injected. A dose of 0.5 cc of Dochez's serum produced a blanched area measuring 7.5 cm in diameter, and a smaller dose of 0.2 cc blanched an area measuring only 4.8 cm in diameter. Sixty hours after the onset of the rash, positive blanching reactions occurred both with convalescent serum and Dochez's serum with a dose of 0.5 cc within 12 hours after the injection, and the area blanched measured 5 cm in diameter with Dochez's serum and only 2.6 cm with the convalescent serum. With a dose of 0.2 cc of convalescent

serum, no blanching reaction was obtained 60 hours after the appearance of the rash, while the same dose of Dochez's serum continued to produce the blanching phenomenon within fourteen hours following the injection, the area blanched measuring 3.5 cm in diameter. Seventy hours after the onset of the rash, similar doses of 0.5 and 0.2 cc of convalescent serum and Dochez's serum were injected intradermally. Injections made with convalescent serum failed entirely to produce the blanching reaction while Dochez's serum produced positive reactions within 17 hours after injections of 0.5 and 0.2 cc doses, the former dose producing a blanched area measuring 4 cm in diameter and the latter dose an area measuring 2.4 cm in diameter. Eighty hours after the onset of the rash tests made with convalescent serum and Dochez's serum were consistently negative.

A noticeable difference was observed in the size of the areas blanched with normal human serum, convalescent scarlet fever serum and Dochez's serum (fig. 1) when the same amount of serum was injected intradermally, in three situations in close proximity one to the other. There appeared simultaneously at the site of the three injections within nine hours three definitely blanched areas regularly edged and circular in shape, but of different sizes. The area blanched by the normal human serum measured 2.2 cm in diameter, the area blanched by the convalescent serum 2.4 cm in diameter and the area blanched by the Dochez serum measured 4.8 cm in diameter. • These tests were repeated sixty hours after the onset of the rash when doses of 0.2 cc were injected intradermally. Fourteen hours following the injection there appeared a definite rash extinction in the area injected with Dochez's serum, regular in outline and circular in shape, measuring 4 cm in diameter. No reaction was noted in the areas injected with normal human serum and convalescent scarlet fever serum.

As was noted by Blake, Trask and Lynch (13) no pigmentation or desquamation was observed during convalescence in the areas blanched within twenty-four hours after the appearance of the exanthem. When the tests were made on the succeeding days of the disease, there was noted during convalescence a faint yellowish-brown discoloration over the blanched area which often desquamated in fine powdery-like fragments before general desquamation occurred.

FIG 1 AREAS OF BLANCHING FOLLOWING INTRADERMAL INJECTIONS OF 0.2 CC OF (TOP) NORMAL HUMAN SERUM, (MIDDLE) CONVALESCENT SCARLETT FEVER SERUM AND (BOTTOM) DOCHEZ'S SCARLATINAL ANTISTREPTOCOCCIC SERUM, NINE HOURS AFTER INJECTION

The areas blanched by Dochez's serum presented a great variety of shapes. Most of them were regular, round or oval and sharply accentuated by the surrounding erythematous skin. Frequently the areas were irregular showing finger-like projections (figs. 2 to 5). The tips of these extensions seemed to fol-

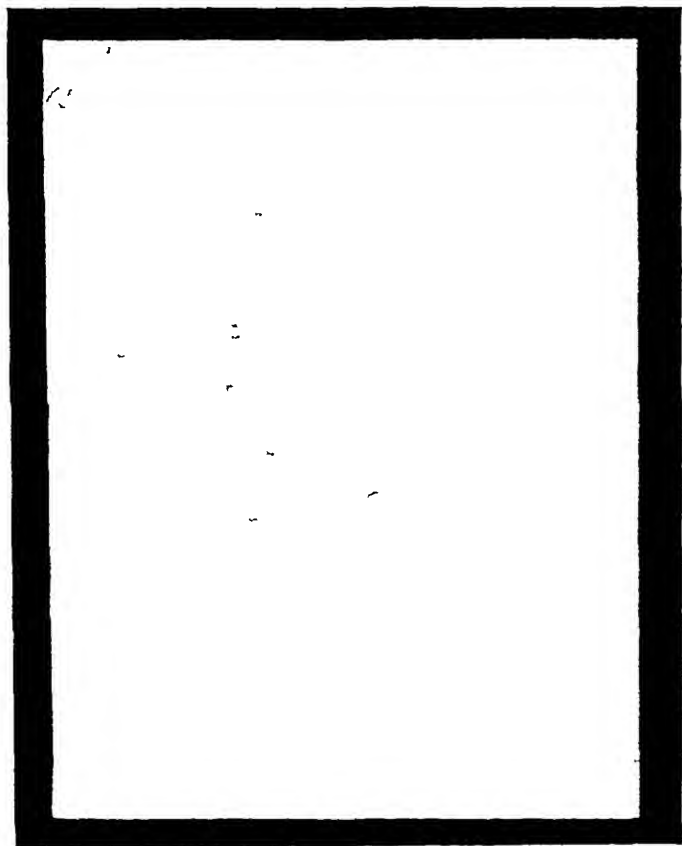


FIG. 2. AREA OF BLANCHING FOLLOWING INTRADERMAL INJECTION OF 0.5 CC. OF DOCHETZ'S SCARLATINAL ANTISTREPTOCOCCIC SERUM FIFTEEN HOURS AFTERWARDS

low the course of the lymphatics and often measured 9 to 12 cm. in their longest diameter. The blanching phenomenon usually reached its height fifteen to twenty-four hours after the injection of the serum. The area of blanching varied from 2.3 to 11.2 cm. in diameter, the size depending directly on the amount of

the serum injected into the skin and the duration of the rash. The shortest interval between the intradermal injection of Dochez's serum and the appearance of blanching was 6 hours and the longest interval 18 hours.

About 80 per cent of the rash extinction tests persisted until the disappearance of the general rash. Fifty-one control tests were made

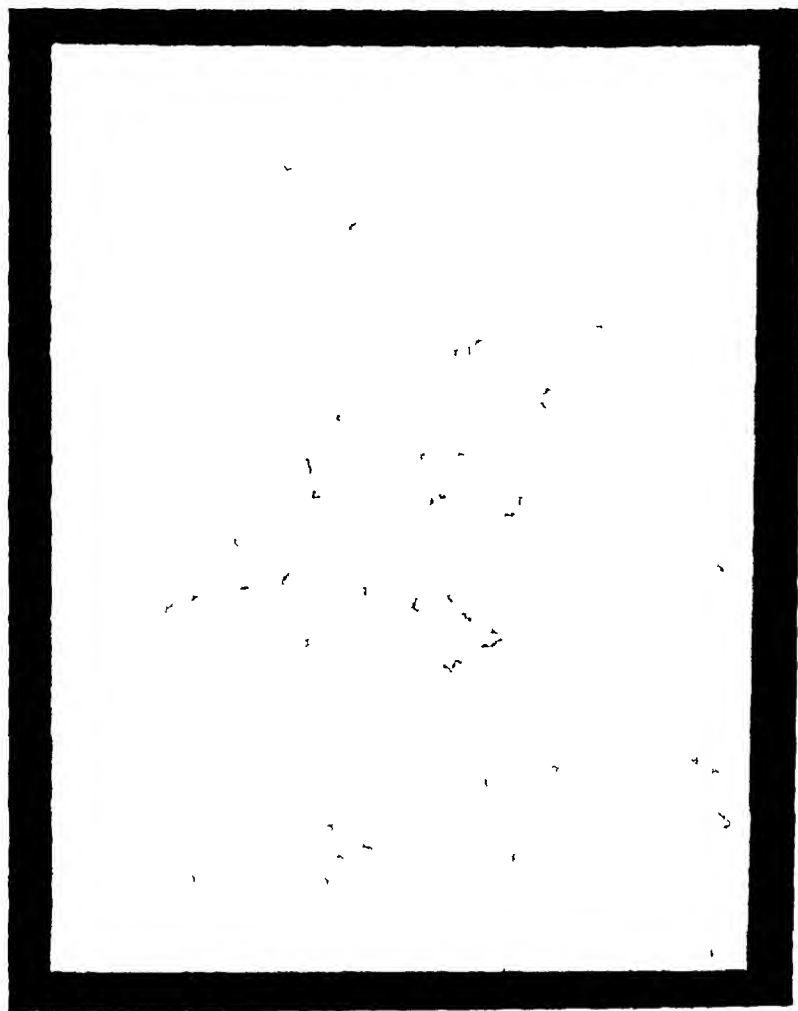


FIG. 3. SAME AREA THIRTEEN HOURS AFTER INJECTION

intradermally with equivalent amounts of diphtheria anti-toxin and normal horse serum. In no instance was the rash blanched by these control sera. Dochez's serum failed to produce the blanching phenomenon in seven cases of erysipelas, four cases of measles and six cases of rubella.

*Pseudo-reaction* A peculiar reaction occurred in case no 12 (table 3) tested intradermally with Dochez's serum seventy hours after the appearance of the exanthem. The amount of serum injected was 1 cc. Twelve hours following the injection, there appeared in the area

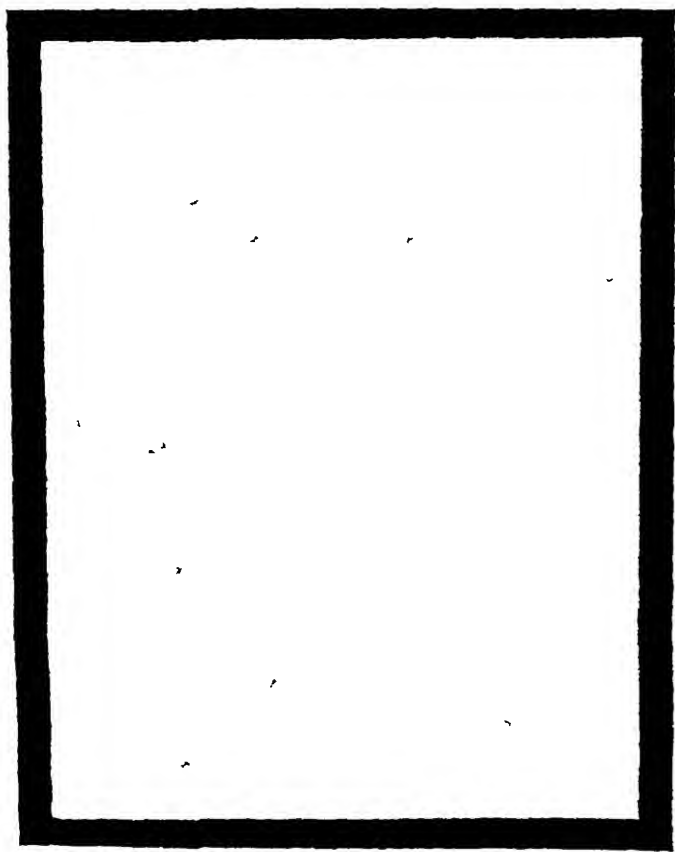


FIG. 4. SAME AREA EIGHTEEN HOURS AFTER INJECTION.

injected an intense erythema followed by marked hyperemia in the underlying skin. The redness rapidly extended in all directions, until twenty-six hours following the injection it attained a diameter of 9.8 cm. with a definite red margin slightly raised above the surface of the surrounding skin. At this time a definite zone of blanching was



observed along the edges of the erythematous area, slowly working its way towards the center of this area. Forty-eight hours after the injection of the serum, the central erythema was faded and the entire area appeared definitely blanched. Vesiculation of the surface epithe-

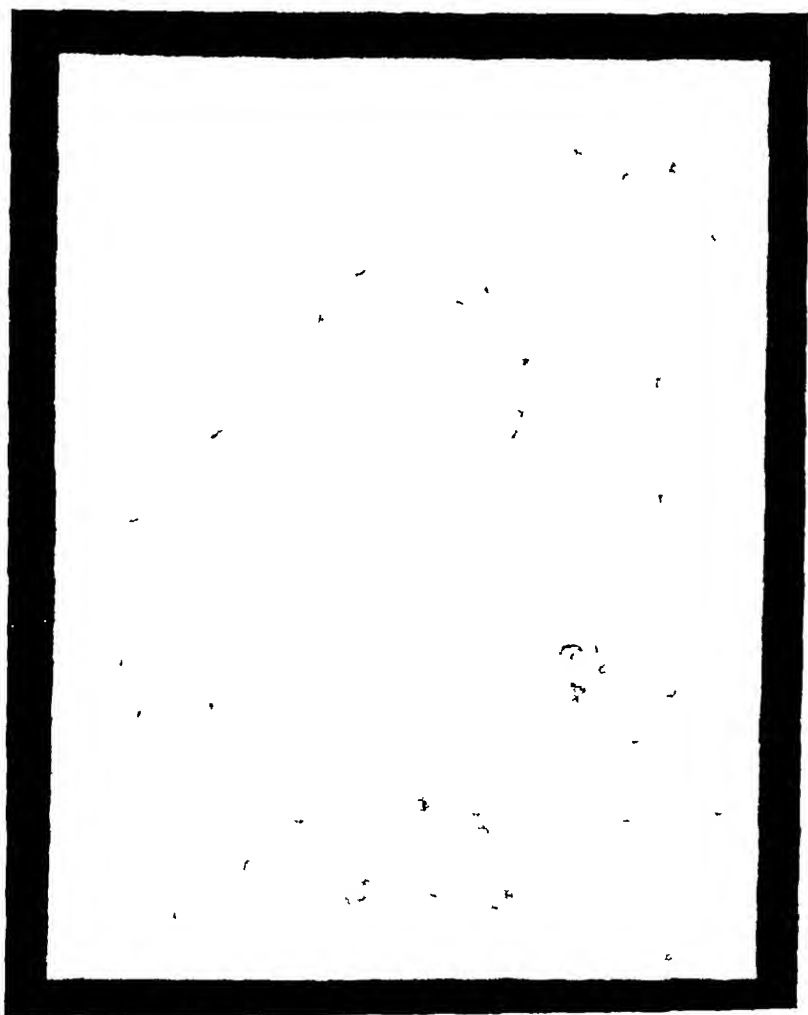


FIG. 5 SAME AREA TWENTY-FOUR HOURS AFTER INJECTION

lium in this area was observed on the following day. No residual pigmentation was noted after the disappearance of the general rash. The combined reaction in this case strongly suggested a local hypersensitiveness to the non-toxic proteins of the horse serum, the effect of which at first prevented the blanching phenomenon.

## DISCUSSION

These observations are confirmatory of previous work and show further that Dochez's antistreptococcic serum possesses similar properties to those contained in normal human serum and convalescent scarlet fever serum. The blanching phenomenon was obtained in this series with great regularity during the first and second day after the appearance of the rash, when intradermal tests were made with normal human serum, convalescent scarlet fever serum and Dochez's serum in scarlet fever patients. An important quantitative difference was noted between strength of normal and convalescent serum and of Dochez's serum, for while normal serum and convalescent serum failed to produce the blanching phenomenon after the second day of the rash, Dochez's serum continued to blanch the exanthem until the fourth day of the rash. It was likewise observed that the blanching phenomenon was best obtained on the second day after the initial appearance of the rash, and rapidly diminished during the third day, until the capacity to blanch the rash was completely lost on the fourth day of the rash. This occurrence, one might venture to explain, is occasioned by an hypothetical specific toxin so fixed in the tissues, following a certain time of exposure to the disease, that it is not readily neutralized by the hypothetical antitoxic substance present in the normal human serum, convalescent scarlet fever serum or Dochez's serum. Considerable importance and therapeutic value may be attached to the difference in potency of blanching properties contained in these three sera in producing the Schultz-Charlton rash extinction phenomenon during the second and third day of the disease, and this difference may serve to establish a rough quantitative measure of specific antitoxic capacities contained in each of these three sera. It is apparent that Dochez's serum not only produces the rash extinction phenomenon later in the disease than the other two sera, but that it also blanches considerable larger areas of rash than does either the normal human serum or the convalescent serum. These results indicate that Dochez's serum contains the same specific substance which is demonstrable in the normal human serum and convalescent scarlet fever serum but that Dochez's serum possesses this specific property in a much greater concentration, than is demonstrable in either normal human serum or convalescent serum.

It was, furthermore, demonstrated that the blood serum of scarlet fever patients acquired the capacity to blanch the rash in scarlet fever about the eighth day after the onset of the disease. This indicates, in so far as may be ascertained by clinical methods, that sufficient antitoxic substance is produced in the body at this stage of

TABLE 4  
*Results of the Schultz-Charlton phenomenon by other workers*

AUTHOR	NUMBER OF CASES	DOSES	CONVALESCENT SERUM	NORMAL SERUM	DOCHEZ'S SERUM
		cc.	per cent	per cent	per cent
Schultz-Charlton (15)	50	0 5-1 0	88		
Harniss, E (16)	19	0 5-1 0	58		
Paschen, E (17)	71	0 5-1 0	87		
Schultz, W (18)		0 5-1 0	100 (2d) 78 (3d) 60 (4d)		
Tron, G (19)	50	0 5-1 0	21		
Hazelhorst, G (20)	50	0 5-1 0	80		
Steinkopf, C (21)	49	0 5-1 0	83 7		
Mulsow, F W (22)	{ 30 20	0 5-1 0 0 5-1 0	53	40	
Rojo, D J (23)	18	0 5-1 0	78		
Raymond, H (24)	72	0 5-1 0	82		
	{ 14 27	0 5-1 0 1 0	21 63	64 81	
Toomey and Nourse (26)	{ 8 8 18	0 5 1 0 1 0	0 50	50 63 100	
Blum, J (27)	10	0 5	60		
Blake, Trask, Lynch (13)	13	0 02-0 5			100
	{ 9 27 57	0 5-1 0 0 2-1 0 0 2-1 0	89	67	100 (60 hours) 40 (70 hours) 0 (80 hours)

the disease to be detected in the circulating blood. It is important to note that this is simultaneous with the onset of convalescence and the return of the blood-picture to normal, which suggests strongly that the toxic substance is neutralized completely by the antitoxic substance, which at this time occurs in such excess in the circulating

blood of the patient that the serum is capable of producing the Schultz-Charlton rash extinction phenomenon upon the scarlet fever rash of other patients

#### SUMMARY

1 Serum from normal persons, without history of scarlet fever or general septic infections, produced the Schultz-Charlton rash extinction phenomenon in four of six cases tested on the second day of the rash and in one of seven cases tested on the third day of the rash

2 Serum from convalescent scarlet fever patients produced the Schultz-Charlton rash extinction phenomenon in twenty-four of twenty-seven cases tested, or 89 per cent, during the first sixty hours of the rash but did not cause blanching seventy hours after the appearance of the rash

3 Dochez's serum produced the Schultz-Charlton rash extinction phenomenon in forty cases, or 100 per cent, during the first sixty hours of the rash. It continued to produce blanching seventy hours after the appearance of the rash, but did not blanch eighty hours after the appearance of the rash. This indicates that Dochez's serum possesses the same specific property that is found in normal and convalescent serum but contains this specific property in considerably greater concentration

4 Blood serum from scarlet fever patients first showed the property of producing the Schultz-Charlton rash extinction phenomenon about the eighth day of the disease

5 Epinephrin, normal horse serum and diphtheria antitoxin failed consistently to produce the Schultz-Charlton rash extinction phenomenon in scarlet fever

6 Normal human serum, convalescent scarlet fever serum and Dochez's serum failed consistently to produce blanching reactions in other eruptive diseases

7 Rash extinction areas were directly proportional in size to the amount of serum injected

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## BLOOD PIGMENTS IN PERNICIOUS ANEMIA

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The conception of pernicious anemia as a disease associated with increased blood destruction has been widely accepted, but the problem of the mechanism involved in the destructive process has given rise to much debate. The subject demands further study and a preliminary note on one phase of the investigations going on in this laboratory has already been published (1). Phagocytosis of red cells in the bone marrow and hemolymph nodes of active cases of pernicious anemia was found to be a striking phenomenon. Observations on the blood pigments and their possible significance in relation to blood destruction will be discussed in the present communication. Particular attention has been directed to the variations in the amount of bilirubin in the blood plasma in different stages of the disease in patients who could be followed over long periods of time.

### METHODS AND TECHNIQUE

Plasma bilirubin was quantitated by the method of van den Bergh and Snapper (2), an adaptation of the diazo reaction of Ehrlich. This procedure, while somewhat more laborious than the usual dilution methods, has the advantage of greater accuracy in that lipochrome, hematin, hemoglobin and alimentary lipemia do not interfere with the quantitation. The technique described by Van den Bergh and Snapper was followed closely with one exception, it was found that better results were obtained with a more concentrated diazo reagent. The reagent used in these observations was made up as follows:

Solution 1	
Sulfanilic acid	4.5 grams
Concentrated HCl	50 cc.
Distilled H <sub>2</sub> O	450 cc.
Solution 2	
Sodium nitrite	5 grams
Distilled H <sub>2</sub> O	500 cc.

Immediately before use, one part of solution 2 is added to fifty parts of solution 1  
In addition to this reagent two other solutions are required

Standard solution of bilirubin

Bilirubin	0.025 grams
Chloroform	100 cc.

Bicarbonate alcohol solution

NaCl	1.5 grams
NaHCO <sub>3</sub> dissolved in 132 cc distilled H <sub>2</sub> O	0.3 grams

When solution is complete 368 cc of 96 per cent alcohol is added

The quantitation is done in the following manner Five cubic centimeters of blood are withdrawn from the patient's vein and placed in a tube containing one drop of 20 per cent potassium oxalate solution The tube is corked, gently inverted several times, centrifuged, and the supernatant plasma removed One cubic centimeter of the latter is measured into a second tube and 2 cc of 96 per cent alcohol added They are mixed by inversion and centrifuged for ten minutes at 2800 r p m Two cubic centimeters of the supernatant fluid are then measured into a tube and 0.5 cc of freshly prepared diazo reagent added Finally 2.5 cc. of 96 per cent alcohol are added to the tube to clear up the fatty acid cloud A purple color develops in the presence of bilirubin and the intensity is read in a colorimeter against the following standard

	"
Bicarbonate alcohol solution	4.9
Standard solution of bilirubin in chloroform	0.1
Diazo reagent	1.25
96 per cent alcohol	6.25

If the unknown solution be set at 10 mm the reading of the standard in centimeters multiplied by 1.1 gives plasma bilirubin in milligrams per 100 cc

In addition to the quantitative method just described, van den Bergh, somewhat later (3) devised the so-called "direct" diazo reaction as a means of distinguishing jaundice due to liver injury from that associated with certain anemias and congenital hemolytic jaundice

The test is performed as follows

To 1 cc of plasma are added 2 cc of distilled water and 0.8 cc of freshly prepared diazo reagent The contents of the tube are at once mixed by inversion and the time of development of a distinct purple color is noted If the purple color is seen within one minute the reaction is "prompt" If it requires more than a minute but still occurs the reaction is spoken of as "delayed" If no purple color appears the reaction is "negative" According to van den Bergh's interpretation a "prompt" reaction denotes jaundice due to liver damage, and a "delayed" reaction, jaundice due to blood destruction

The majority of the hemoglobin determinations were made by the method of Newcomer (4) The color value of the glass standard was controlled by reading

against it a number of specimens of blood the hemoglobin content of which had been determined by the Van Slyke (5) oxygen capacity method. The hemoglobin readings given are in terms of the Haldane scale. On this scale a hemoglobin reading of 100 per cent corresponds to an oxygen carrying capacity of 18.6 cc. and a hemoglobin content of 13.8 grams per 100 cc. of blood. Some of the earlier determinations were made by the Sahli method. Red blood cell counts were made in duplicate with Hayem's fluid as the diluting medium.

Examinations for hemoglobin and hematin in the plasma were made with a small spectroscope.

Lipochrome was extracted from the plasma by shaking with ether and this extract was used in subsequent examinations.

#### PLASMA BILIRUBIN VALUES IN PERNICIOUS ANEMIA AND VARIOUS OTHER ANEMIAS

Increases in the plasma bilirubin in cases of pernicious anemia have been reported by van den Bergh and other observers (6) (7). A series of such observations on 28 cases is shown in table 1. A large number of observations, made with the same technique and standards, on normal individuals and unjaundiced hospital patients have given values varying between 0.1 and 0.7 mg. of bilirubin per 100 cc. of plasma. The cases of pernicious anemia, therefore, show increases which in some cases are slight, (e.g. cases 16, 17, 20 and 22) and in other instances are well marked (10, 12, 13, 14, 15, 19, 24, 25, 26, 27 and 28). Cases 1, 2, 3 and 6 show only moderate increases but these, at the time of observation, were clinically in a stage of remission. It is evident that there is little direct relation between the quantity of bilirubin present in the plasma and the level of the red count of different cases.

It will be noted that the direct diazo reaction is always "delayed." In no instance was a "prompt" direct diazo reaction obtained during life on a case clinically typical of pernicious anemia. Two specimens of plasma obtained several hours post mortem gave "prompt" direct reactions but a terminal liver lesion could not be definitely ruled out.

The increase in plasma bilirubin in pernicious anemia is often of diagnostic importance. Table 2 gives the findings in cases of secondary anemia due to various causes. Anemias accompanying nephritis, tuberculosis, acute or chronic hemorrhage, and malignant tumors give normal or low plasma bilirubin values except in the



cases where there is jaundice due to a liver or bile duct lesion. In cases 40 and 41, where carcinoma involved the bile ducts, this condition prevailed and the jaundice could easily be distinguished from that of pernicious anemia by the occurrence of a "prompt" direct reaction

TABLE 1  
*Plasma bilirubin in pernicious anemia*

Case number	Red corpuscles	Hemoglobin (Haldane Scale)	"Direct" diazo reaction	Plasma bilirubin
	<i>millions per cu mm</i>	<i>per cent</i>		<i>mg per 100 cc</i>
1	4 128	105	Delayed	1 1
2	2 392	76	Delayed	0 8
3	2 136	59	Delayed	1 0
4	1 992	50	Delayed	2 2
5	1 840	50	Delayed	1 2
6	1 704	61	Delayed	1 1
7	1 600	38	Delayed	1 8
8	1 500	42	Delayed	2 0
9	1 420	30	Delayed	1 7
10	1 208	35	Delayed	2 2
11	1 200	24	Delayed	1 5
12	1 192	35	Delayed	2 0
13	1 072	30	Delayed	2 3
14	1 028	35	Delayed	2 0
15	0 936	30	Delayed	3 3
16	0 928	35	Delayed	1 0
17	0 888	26	Delayed	1 0
18	0 850	20	Delayed	1 3
19	0 848	22	Delayed	3 4
20	0 824	22	Delayed	1 0
21	0 656	19	Delayed	1 9
22	0 640	14	Delayed	1 0
23	0 592	23	Delayed	1 9
24	0 584	17	Delayed	2 3
25	0 569	16	Delayed	2 5
26	0 480	17	Delayed	4 0
27	0 472	12	Delayed	2 8
28	0 456	10	Delayed	2 5

Other types of anemia, however, as shown in table 3, may give plasma bilirubin findings which are indistinguishable from pernicious anemia. Case 42, with a history suggestive of congenital hemolytic jaundice, showed a high reticulated red cell count, a marked anemia, and a plasma bilirubin distinctly above normal, which gave a "de-

TABLE 2  
*Plasma bilirubin in secondary anemia*

Case number	Red corpuscles	Hemo-globin (Haldane Scale)	"Direct" diazo reaction	Plasma bilirubin	Diagnosis
	millions per cu mm.	per cent		mg per 100 cc.	
29	1 500	32	Negative	0	Chronic nephritis
30	2 128	25	Negative	0	Chronic nephritis
31	4 360	63	Negative	0 3	Pulmonary tuberculosis
32	2 640	60	Negative	0 1	Pulmonary tuberculosis
33	5 272	42	Negative	0 3	Bleeding hemorrhoids
34	2 360	33	Negative	0 2	Bleeding hemorrhoids
35	1 264	30	Negative	0 1	Bleeding duodenal ulcer
36	3 672	38	Negative	0 1	Banti's disease. Gastric hemorrhage
37	2 672	45	Negative	0 3	Primary carcinoma of kidney, with liver metastases, not obstructing the larger bile ducts
38	1 820	21	Negative	0 1	Carcinoma of sigmoid
39	2 792	51	Negative	0 1	Rhabdomyosarcoma involving pleura, pericardium and other thoracic structures
40	2 880	25	"Prompt"	2 0	Carcinoma of stomach with liver metastases
41	3 170	48	"Prompt"	9 6	Carcinoma of head of pancreas involving the common bile duct

TABLE 3  
*Anemias with plasma bilirubin, findings similar to pernicious anemia*

Case number	Red corpuscles	Hemo-globin (Haldane Scale)	"Direct" diazo reaction	Plasma bilirubin	Diagnosis
	millions per cu mm.	per cent		mg per 100 cc.	
42	2 000		Delayed	2 7	Familial hemolytic jaundice
43	1 992	58	Delayed	1 2	Sprue
44	2 552	58	Delayed	1 3	Anemia following alcoholic intoxication
45	3 808	50	Delayed	2 0	Lymphatic leukemia
46	2 208	40	Delayed	0 5	Post partum anemia
47	1 174	24	Delayed	0 8	Subacute bacterial endocarditis
48	2 690	49	Delayed	0 9	Subacute bacterial endocarditis
49		82	Delayed	1 0	Typhoid fever
50	4 840	112	Delayed	1 1	Lobar pneumonia

layed" direct diazo reaction Case 43, of tropical sprue, contracted in the Phillipine Islands, presented a blood picture very similar to pernicious anemia and the plasma bilirubin findings are of the same type. Case 44, apparently developed an acute hemolytic anemia after an alcoholic debauch on liquor of doubtful quality From this anemia he quickly recovered, and the blood bilirubin at the same time returned to normal limits A case of lymphatic leukemia (no 45) gave high blood pigments but a blood picture typical of the leukemic condition The post-pregnancy anemia (no 46) is listed here because, while giving normal pigment values, the case resembled pernicious anemia closely in other respects Subacute bacterial endocarditis, typhoid fever, and pneumonia may at times give slightly increased blood bilirubin as shown in cases 47, 48, 49 and 50 It should be mentioned here that some cases of pneumonia and streptococcus sepsis with jaundice, give a "prompt" direct diazo reaction In such cases focal necrosis of the liver probably occurs as a complication The evidence indicates, however, that anemias of the general types included in this table, while differing widely in their clinical and pathological pictures, may all be associated with an increase of blood destruction

#### VARIATIONS IN PLASMA BILIRUBIN AT DIFFERENT STAGES OF PERNICIOUS ANEMIA

The chief value of the present study depends on the fact that it was possible to make numerous observations on many of the patients over long periods of time and at different stages of the disease Repeated determinations of plasma bilirubin in individual cases of pernicious anemia show considerable fluctuations which usually bear a distinct relation to the clinical condition of the patient The onset of a period of diarrhoea or a distinct increase in pallor and weakness are often accompanied by a rise in plasma bilirubin above the previously existing level for that patient When followed over a period of months a certain general reciprocal relation between the bilirubin curve and that of the erythrocyte counts and hemoglobin will be seen During periods when the red cell count and hemoglobin are decreasing, a rise in blood bilirubin is noted, while an increasing red count is usually accompanied by a return of the plasma pigment

TABLE 4

Case number	Day of observation	Red corpuscles	Hemoglobin (Haldane Scale)	Plasma bilirubin	Remarks
		<i>millions per cu mm.</i>	<i>per cent</i>	<i>mg per 100 cc</i>	
1	1	4 726	86	0 9	
	14	4 768	87	0 6	
	32	5 592	102	0 4	
	52	4 640	96	0 4	
2	1	2 232	88	0 4	
	10	2 520	89	0 6	
	35	2 392	76	0 8	Diarrhoea
	49	2 280	69	0 6	
	85	2 272	67	0 3	
	111	3 088	90	0 5	
	122	2 992	84	0 5	
	136	3 616	88	0 3	
	173	3 776	98	0 4	
	179	4 088	97	0 4	
	189	4 184	93	0 4	
3	1	3 364	66	0 3	
	8	3 600	79	0 2	
	87	3 552	64	0 4	
	98	3 936	60	0 8	
	143	3 724	80	0 6	
	270	2 136	59	1 0	
					Reticulated red corpuscles
					<i>per cent</i>
6	1	1 256	45	0 8	2 1
	3	0 928	34	0 8	0 1
	15	0 744	25	0 8	0 7
	25	1 264	35	0 6	0 9
	36	1 328	34	0 7	4 0
	44	1 336	67	0 6	3 3
	57	1 248	55	0 5	3 1
	63	0 888	45	0 4	
	72	1 264	49	0 6	3 1
	83	1 504	47	0 6	2 4
	99	1 660	59	0 5	2 5
	114	1 656	45	0 9	1 4
	127	2 048	71	1 2	6 5
	156	1 138	38	0 6	3 3
15	1	1 624	46	1 1	0 2
	17	1 464	46	1 1	1 2
	39	1 448	40	1 4	0 8

TABLE 4—Continued

Case number	Day of observation	Red corpuscles	Hemoglobin (Haldane Scale)	Plasma bilirubin	Remarks
					Reticulated red corpuscles
		millions per cu mm	per cent	mg per 100 cc	per cent
15	54	1 424	34	1 4	1 0
	67	1 256		2 4	0 6
	73	0 920	28	2 5	0 2
	81	0 952	23		7 3
	85	1 400	36	2 5	7 1
	92	0 952	35	1 6	2 2
	96	1 240	43	1 1	3 7
	109	1 456	45	1 5	3 4
	123	1 432	41	1 6	2 1
	135	1 000	34	2 3	2 9
	149	0 928	36	2 0	1 8
	164	0 936	30	3 3	0 5
	178	1 120	38	2 4	2 4
4	191	0 888	29	3 2	0 2
	201	1 096	33	2 4	2 4
	1	3 744	73	0 9	
	34	4 055	72	0 9	
	60	2 808	60	1 3	Developed lobar pneumonia
	63			2 2	
11	64	1 992	50	1 7	
	65	2 120	49	0 8	Died
	1	2 040	44	0 9	
	14	1 320	25		Developed streptococcus hemolyticus septicemia from acute otitis media
	15	1 200	24	1 5	
	17	0 870	13	0 6	Transfused
	19	1 171	26	1 3	
	21	0 805	21	1 3	Died
	1	0 848	22	3 4	
	2				Transfused
	4	1 080	27	1 7	
19	8	1 104	24		Transfused
	17	1 294	27	1 4	
	19	1 508	30		Transfused
	21	1 640	35		
	31	1 216	28	1 1	
	42	0 744	18	1 3	

TABLE 4—Continued

Case number	Day of observation	Red corpuscles	Hemoglobin (Haldane Scale)	Plasma bilirubin	Remarks
		<i>millions per cu mm.</i>	<i>per cent</i>	<i>mg per 100 cc.</i>	
23	1			1.5	
	2	0.840	35	1.5	
	10	0.592	23	1.9	Transfused
	12	1.496	38	1.2	
	19	1.304	43	1.0	
	22	1.680	50	0.85	
	33	1.560	30	1.0	
	46	1.080	27	1.9	
	52	0.956	25	1.9	Transfused
	60	1.388	45	1.3	
24	1	0.584	17	2.3	Transfused
	2	1.280	26		
	7	0.724	19		Transfused
	12	1.336	30	1.3	
	22	1.152	34	0.7	
	35	0.664	22	1.3	Transfused
	47	1.272	28	1.2	
26	1	1.944	61	0.7	
	11	2.296	78	0.8	
	22	2.320	64	0.6	
	48	0.968	42	0.8	
	56	0.736	32	1.0	
	64			1.7	
	67	0.600	20	1.2	
	70	0.416	14	2.8	Transfused
	76	1.824	35	1.4	
	88	4.000	79	0.4	
	98	3.288	69	0.6	
	109	2.520	94	0.6	
	117	2.696	84	0.6	
	120	2.496	88	0.5	
	130	3.504	67	0.5	
	138	2.976	78	0.4	
	151	2.360	66	0.4	
	175	1.728	59	1.0	
	184	1.408	37	1.3	Transfused
	187	0.656	35	1.25	
	198	0.680	19	1.20	
	201	0.480	17	4.00	Died

TABLE 4—*Concluded*

Case number	Day of observation	Red corpuscles	Hemoglobin (Haldane Scale)	Plasma bilirubin	Remarks
		millions per cu mm	per cent	mg per 100 cc	
28	1	1 000	18	2 0	Transfused Transfused
	19	0 804	16	2 2	
	22	1 472	22	0 4	
	37	3 328	60	0 3	
	49	2 168	68	0 2	
	57	2 704	60	0 3	
	218	0 888	23	1 8	
	228	0 592	15	2 0	
	229	0 456	10	2 5	

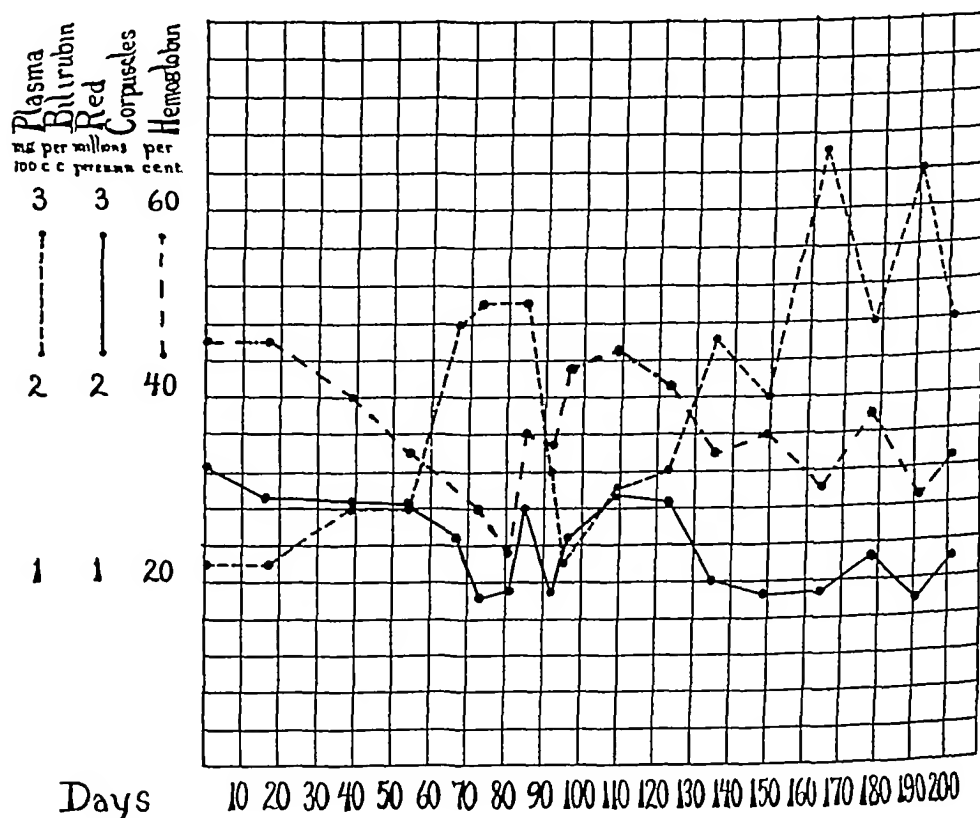


FIG 1 CASE 15 VARIATIONS IN PLASMA BILIRUBIN, RED BLOOD CORPUSCLES, AND HEMOGLOBIN

to more nearly normal levels. As might be expected, occasional exceptions to this generalization are met with.

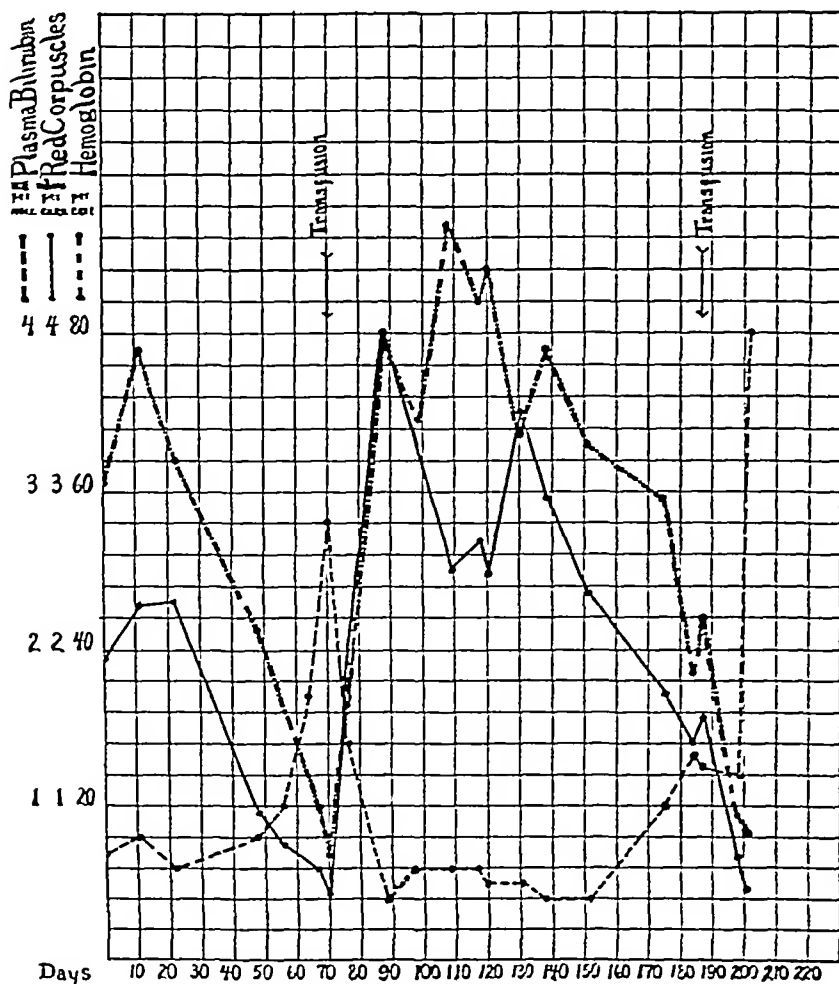


FIG 2 CASE 26 VARIATIONS IN PLASMA BILIRUBIN, RED BLOOD CORPUSCLES, AND HEMOGLOBIN.

The findings in a number of typical cases are shown in the accompanying table (table 4) and charts (figs 1, 2, 3 and 4)



Case 1 (table 4) was at one time a severe case of pernicious anemia but has now been in remission for several years. The plasma bilirubin values are at times normal, at times slightly elevated.

Case 2 (table 4) never showed a very active hemolytic process. Neurological symptoms always predominated. A small rise in plasma bilirubin occurred at one

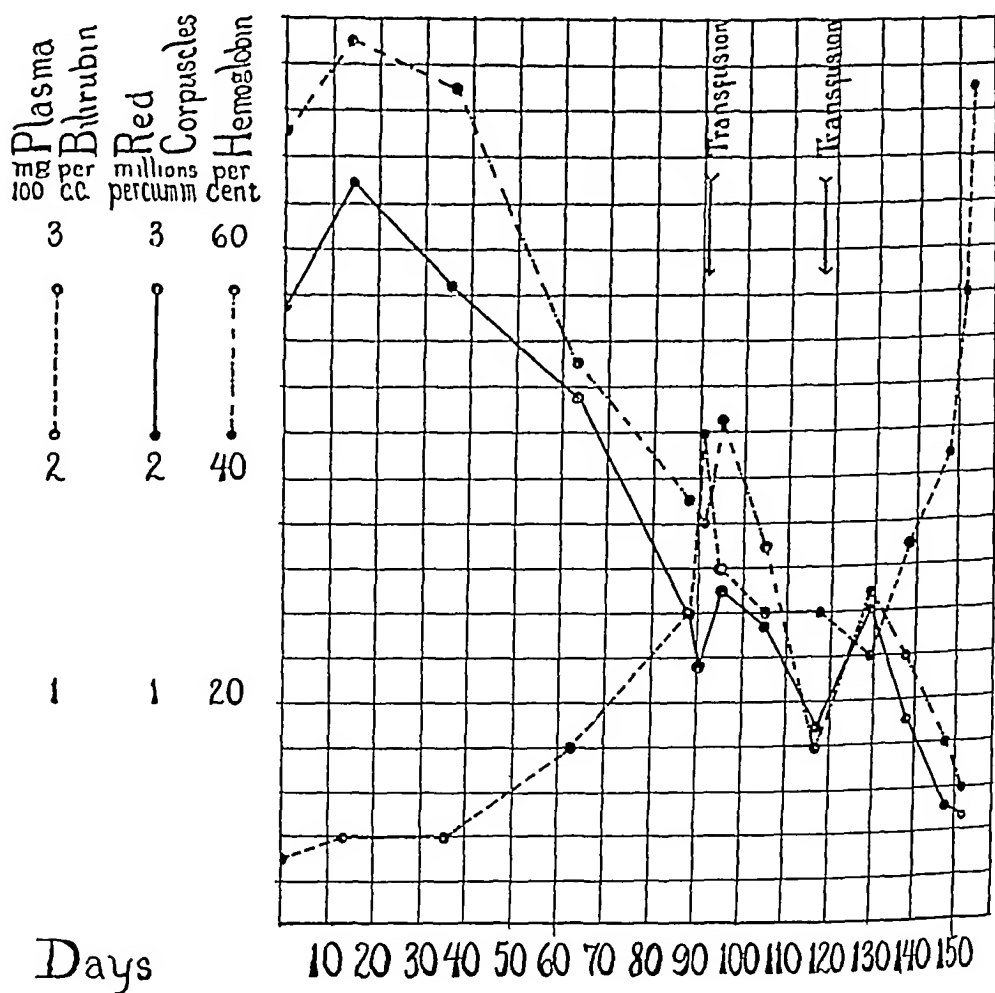


FIG 3 CASE 27 VARIATIONS IN PLASMA BILIRUBIN, RED BLOOD CORPUSCLES, AND HEMOGLOBIN

period marked clinically by severe diarrhoea, and accompanied by a very slight decrease in red count and hemoglobin. The low bilirubin value on the 85th day was indeed observed at the time when the hemoglobin was lowest, but it marked the beginning of a period of clinical improvement which has continued without interruption to the present time.

Case 3 (table 4) had suffered from a severe anemia but these observations were

made during a period of remission. It is interesting that at times a low color index was found. The final observations seemed to indicate a return of the anemia accompanied by an increase of plasma bilirubin. The case did not return for observation after this time.

Case 6 (table 4) presents more variation from the usual trend of the curves than has any other case so far observed. He entered with a severe grade of anemia and during the period of observation went into a partial remission. On the 127th day of observation a distinct rise in pigments is noted. This occurred, contrary to the usual rule, during a period of apparent improvement. A marked rise in reticu-

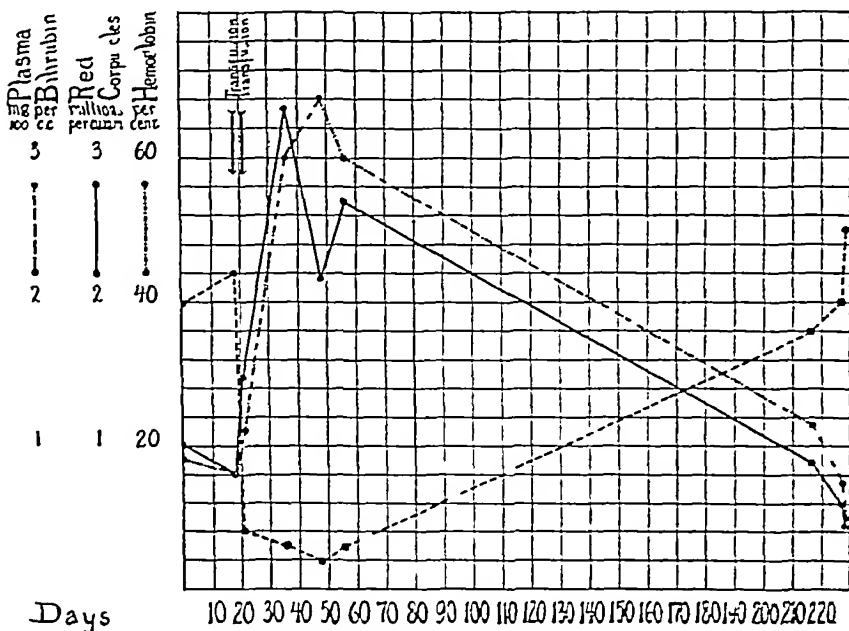


FIG 4 CASE 28 VARIATIONS IN PLASMA BILIRUBIN, RED BLOOD CORPUSCLES AND HEMOGLOBIN

lated cells occurred at this time and it is conceivable that many of these immature cells were quickly destroyed. The patient was not in the hospital but was reporting once in two weeks as an out-patient.

Case 15 (table 4 and fig 1) illustrates a far more active process than that observed in the four preceding cases. Here a distinct exacerbation of the anemia reduced the red cell count from 1,624,000 to 920,000 and the hemoglobin from 46 to 23 per cent. During the same period the plasma bilirubin rose from 1.1 mg per 100 cc to 2.5 mg. A spontaneous remission followed during which a fall in blood bilirubin occurred. During the succeeding months the fluctuations, particularly

in hemoglobin, are distinctly reflected in the bilirubin curve. The case has now been followed for nearly two years and general relationship between these curves has remained comparatively constant. An exception occurred on the 85th day of observation when the plasma pigment remained high although a period of regeneration had already begun. As was noted in similar circumstances in case 6, the reticulated red cell count was quite high and the same hypothesis may be advanced, namely that the presence of immature cells in large numbers may have increased the proportion of casualties in the circulation.

#### EFFECTS OF TRANSFUSION

One of the interesting features of the plasma bilirubin curves is the decrease in circulating bilirubin which usually takes place after transfusion. This decrease may be immediate or may go on gradually for two weeks or more. If it fails to occur the beneficial effects of the transfusion are frequently less evident than in instances giving a distinct reduction.

In cases 19, 23 and 24 there is very little decrease in plasma bilirubin but there was a rapid disappearance of the blood given at transfusion and only slight temporary benefit was received.

Case 26 (table 4, fig 2) is particularly striking in that it shows opposite effects after two transfusions. A transfusion on the 70th day of observation was followed by a marked remission and a fall in plasma bilirubin. The next transfusion, however, given on the 184th day, had very little clinical effect and death followed seventeen days later during a period of marked rise in the plasma bilirubin curve. Case 28 illustrates again the fall in bilirubin after a successful transfusion.

#### EFFECT OF INTERCURRENT INFECTION

The plasma bilirubin is often affected by the development of an intercurrent infection in a patient with pernicious anemia.

Case 4 (table 4) developed lobar pneumonia during a period of partial remission from his anemia. At the onset a sharp fall in the red corpuscle count and hemoglobin and a sharp rise in the blood bilirubin occurred. No further fall in the blood count occurred as the pneumonia progressed, and the plasma bilirubin had returned to a practically normal level at the time of death which occurred on the sixth day after pneumonia developed.

Case 11 (table 4) developed an acute otitis media and streptococcus hemolyticus septicemia during a period in which the blood count had been rising. At the onset a marked drop in red corpuscle count and hemoglobin and a sharp rise in plasma bilirubin was found. Later, in spite of a continued change for the worse in blood condition, a decrease in plasma bilirubin occurred. At this point a transfusion was given. Very little benefit was received and there followed a distinct rise in the bilirubin curve. Death occurred a few days later from the septicemia.

TABLE 5

Patient number	7.30 a.m.	8.00 a.m.	12.00 m	12.30 p.m.	4.00 p.m.	Diagnosis
	Plasma bilirubin		Plasma bilirubin		Plasma bilirubin	
	mg per 100 cc.		mg per 100 cc.		mg per 100 cc.	
50	0.7	Breakfast	0.5	Dinner	0.4	Normal
51	1.0	Breakfast	0.7	Dinner	0.5	Normal
52	0.3	Breakfast	0.3	Dinner	0.3	Arterial hypertension
53	0.1	Breakfast	0.0	Dinner	0.0	Arterial hypertension
54	0.7	Breakfast	0.4	Dinner	0.5	Neuritis
7	1.4	Breakfast	1.3	Dinner	1.2	Pernicious anemia
13	1.5	Breakfast	0.7	Dinner	1.1	Pernicious anemia
15	2.2	Breakfast	2.5	Dinner	2.5	Pernicious anemia
17	0.4		0.5		0.7	Pernicious anemia
25	2.8		2.4		2.5	Pernicious anemia

## DAILY VARIATION IN PLASMA BILIRUBIN

In order to gain some idea of the variation in plasma bilirubin which occurs in the course of a single day, several normal individuals and cases of pernicious anemia were examined three times in the course of a day. A regular dietary regimen was followed, which included a fairly high-fat breakfast and a fat-free meal at noon. The results obtained are listed in table 5.

The cases, with three exceptions, show a slightly higher value for the fasting specimen than for the specimens taken four and eight hours after the fat meal. While the variations during the day are in many instances well marked they are not sufficient to account for the changes which were observed at different stages of the anemia.

The specimens included in tables 1, 2, 3 and 4 were taken in the period between breakfast and dinner corresponding approximately to the second specimen of table 5

#### PIGMENTS OTHER THAN BILIRUBIN IN THE PLASMA OF CASES OF PERNICIOUS ANEMIA

Brockbank (8) has noted the constant occurrence of hemoglobin on spectroscopic examination of the plasma of cases of pernicious anemia With this observation we are in entire accord It is difficult to be sure that the hemoglobin present in plasma is not due to trauma in collecting blood but as the same technique was used in every instance this error is probably negligible No effort at direct quantitation was made, but the impression was received that in general more distinct spectroscopic bands appeared during periods of clinical exacerbation than during periods of remission

Bands of hematin were occasionally seen—an observation which van den Bergh (9) has also reported This pigment is usually seen during periods of marked activity of the disease

Another pigment found in severe cases during periods when the blood count was very low probably belongs to the general class of lipochromes It is readily extracted from the plasma by ether and does not give the diazo reaction However, on testing it with various reactions described for lutein by van den Bergh (2), entirely negative results were obtained The exact nature of this pigment remains uncertain It appears chiefly in severe exacerbations of anemia, particularly just before death, and it is present in large quantities post mortem

#### DISCUSSION

The significance to be attached to the foregoing observations on plasma bilirubin is inevitably bound up with the question of the origin of this pigment Stadelman (10) has held that bilirubin is produced only in the liver and that all jaundice is of hepatic origin This theory is based on the observations of Minkowski and Naunyn (11) on hepatectomized geese Whipple and Cowper (12) offered the first direct evidence against it by their experiments with a thoracic circulation in mammals Mann and his co-workers (13) have proba-

bly finally settled the point by their observations on liver ablation in dogs. A short time after complete removal of the liver their animals became as deeply jaundiced as though the common bile duct had been ligated. Jones (14) has shown the occurrence of a local increase of bilirubin in the peripheral vessels of man during an attack of paroxysmal hemoglobinuria. It has long been known that substances indistinguishable from bilirubin are found in blood extravasations into the body tissues or into the pleural or peritoneal cavities. Therefore, the evidence at present available strongly supports the view that bilirubin can be formed outside of the liver and for pigment formed in this manner the liver acts merely in an excretory capacity. The most obvious source from which this bilirubin could be derived is the hemoglobin set free in the process of red cell destruction. Whipple (15), while admitting that this process occurs, has contended that the quantity of bilirubin excreted by the liver can be altered by the type of food eaten, and that carbohydrate feeding causes an increase in bile pigment. Rous, Broun and McMaster (16), on the other hand, were unable to find any increase in bilirubin excretion on a carbohydrate diet when the entire twenty-four hour output was collected over a considerable period of time. Diets which contain bilirubin, however, do increase the amount of pigment excreted by the liver (17). Our data regarding the plasma bilirubin indicate that the pigment is usually present in greater quantity in fasting periods than during the process of absorption after meals. It seems possible, therefore, to exclude dietary factors and to assume that the plasma bilirubin was derived from hemoglobin in the process of blood destruction.

The fact that the plasma bilirubin of patients with pernicious anemia always gives a "delayed" direct diazo reaction supports the assumption that in this disease the pigment is not of hepatic origin. Hepatic diseases, and particularly those conditions causing obstruction of the larger bile ducts or extensive degeneration of the parenchyma are of course the most common causes of increased plasma bilirubin. Whenever lesions of this type are found, a "prompt" direct diazo reaction is obtained. While the chemistry underlying this reaction is not clear, the clinical value of the test has been recognized by

several investigators (7) (18) and in our hands it has given satisfactory results in a large series of cases

Three theories must be considered as possible explanations of the cause of the increased plasma bilirubin in pernicious anemia. First, the increase might be due to a failure of the liver to excrete the circulating bilirubin. The objections to this theory are that the output of pigment in the stools is actually increased (19) and that the estimation of liver function by the method of Rosenthal (20) gives normal results in cases of pernicious anemia. Second, a failure of the bone marrow to utilize the pigment in the formation of new red cells might also account for its accumulation in the peripheral circulation. This is extremely unlikely, however, for in secondary anemias and in definitely aplastic anemias no such accumulation of bilirubin in the plasma takes place. The third theory holds that the increase of plasma bilirubin is due to an overproduction which results from an increased rate of blood destruction. This would seem to be the best explanation of the phenomenon at present available, since it accounts for the high plasma pigments, the high stool pigments, and the rapid fluctuations in circulating red cells that are characteristic of the disease. According to this view, the liver, while capable of excreting a normal or even increased quantity of pigment, is unable to eliminate all of the bilirubin that is set free in the plasma in times of very active destruction. The observations reported in this paper extend over considerable periods of time in individual cases of pernicious anemia and the evidence they bring out is also in harmony with this explanation. Similar but less extensive observations have been reported by Gram (6). Periods marked by a falling red cell count and hemoglobin curve generally show increased blood pigment values. Periods when the blood pigments are at more nearly normal levels are usually periods of distinct clinical improvement. The occasional exceptions to this rule are in themselves enlightening. The onset of a remission marked by high reticulated red cell counts and a rising red cell and hemoglobin curve sometimes shows a high blood bilirubin level. This merely indicates that destruction and regeneration are independent processes for it is obvious that if regeneration were sufficiently active, it could counteract any amount of destruction and maintain the blood count at normal levels. This is probably what

happens in cases of hemolytic jaundice without anemia. In pernicious anemia, on the other hand, either the factor of bone marrow exhaustion must come into play or the destructive process must exceed the margin of safety of the bone marrow.

The changes in plasma bilirubin that occur after transfusion are more difficult to explain. It is evident that immediately after transfusion the intermixture of normal plasma with that of the patient causes a dilution of the plasma bilirubin. Even granting that this occurs in every case, however, it fails to account for the marked decrease in plasma bilirubin that is sometimes seen. Neutralization of a circulating hemolysin by an antihemolysin in the transfused blood may also be suggested as a possible explanation, but the reduction in bilirubin often occurs more gradually than would be expected if this were the process at work. Moreover, case 21 received a transfusion of cells suspended in saline on the 28th day of observation and the reduction of plasma bilirubin following this transfusion was just as great as that following two whole blood transfusions given on the 86th and 158th days respectively. If there were a circulating antihemolysin in the blood given to this patient, it must have been contained in the red corpuscles. In some cases there is a suggestion that the destructive process is actually diminished by the transfusion but in other instances the red corpuscle count and the hemoglobin curve fall rapidly after transfusion and yet the bilirubin remains at a lower level than before the transfusion. An increased utilization of pigment by the bone marrow in the formation of new erythrocytes would explain those cases which go into a distinct remission, but this theory fails to account for the instances in which the pigment decreases in spite of lack of evidence of bone marrow activity. Temporary increase in the ability of the liver to excrete pigment, owing to the improved condition of the blood, must also be taken into consideration. The level of bilirubin in the plasma is of course dependent upon the efficiency of the liver as an organ of elimination, for an organ of unlimited capacity for excretion could prevent accumulation of pigment regardless of its rate of formation. It is the balance between rate of formation and rate of excretion which determines the amount of pigment present in the plasma.

The observations on the influence of intercurrent infection on the



plasma bilirubin in pernicious anemia are so few that they do not afford a basis for the explanation of the process at work

The occurrence of hemoglobin and hematin in the circulating plasma indicates that all of the pigment of the destroyed cells is not broken down to the form of bilirubin before being set free in the plasma

The nature of the ether soluble pigment present in the plasma of cases of pernicious anemia has not been determined. Its presence is a bad prognostic sign. At times it occurs in quantities equal to one-third of the dilution value of the plasma pigments. In these cases it is obviously fallacious to conclude that the dilution value represents the quantity of bilirubin present

#### SUMMARY

High values for plasma bilirubin occur constantly in pernicious anemia during periods when the disease is active. They are of considerable diagnostic aid in distinguishing this disease from certain secondary anemias.

Certain cases of pneumonia, streptococcus septicemia, typhoid fever, tropical sprue, and hemolytic jaundice give plasma bilirubin findings similar to those met with in pernicious anemia.

The regular occurrence of a "delayed" direct diazo reaction in pernicious anemia serves to distinguish it from anemias associated with jaundice due to liver or bile duct lesions. In the latter conditions the plasma bilirubin gives a "prompt" direct diazo reaction.

The plasma bilirubin curve in pernicious anemia usually rises during periods of exacerbation of the clinical symptoms, and falls to more normal levels during periods of remission. This phenomenon tends to support the view that pernicious anemia is associated with increased blood destruction.

Following transfusion a reduction in the plasma bilirubin is generally observed. The explanation of this phenomenon is not clear. It would seem to be due either to a slowing of the destructive process, to an increased utilization of the pigment, or to a temporary increase in the ability of the liver to excrete pigment. Hemoglobin and hematin may occur in the plasma of cases of pernicious anemia and they are usually found in periods of clinical exacerbation of the disease. Their presence also suggests red cell destruction.

An ether soluble pigment may also be present in the plasma of cases of pernicious anemia. When found in any considerable quantity it constitutes a serious prognostic sign. It occurs in largest amount post mortem.

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# THE OPTICAL ACTIVITY OF GLUCOSE AS INFLUENCED BY NORMAL AND DIABETIC URINE

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## INTRODUCTION

There have been several recent studies upon the influence which tissues and certain body fluids may exert upon the optical rotatory properties of various sugars. More than one investigator in this field has noted that certain changes occur in the optical rotation of glucose which has been brought into contact with body tissues or fluids, and has suggested that these changes may represent an important step in the preparation of this sugar for its utilization by the body.

The first recent important observation was made by Admont Clark (1), who found that on perfusion of the dog's pancreas with Locke's solution containing glucose in approximately physiological quantities the optical activity of this solution became slightly diminished, but its copper reducing power was unaltered. However, after acid hydrolysis this loss in optical activity was partially regained. No change in the optical activity was noted as the result of similarly perfusing the heart, spleen or kidneys. Furthermore, osazones were obtained from the pancreatic perfusate which had slightly lower melting points than glucazone but approached that of glucazone after acid hydrolysis. Clark concluded that these phenomena were due to an enzyme or enzymes obtained from the perfused pancreas which exerted a specific action on glucose, and was responsible for certain essential steps by which glucose was prepared for utilization by the body.

Another interesting set of experiments somewhat along the same line have been reported by Hewitt and Pryde (2). These observers have described the polarimetric changes occurring in glucose solu-

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tions which have been allowed to remain in contact with the intestinal mucosa of the rabbit for a few minutes. Following this exposure they observed mutarotary phenomena in the glucose solutions which consisted in a rapid diminution of the optical rotation of the sugar to values much lower than that of normal equilibrated glucose. Following the withdrawal from the intestine the solutions underwent a slower dextro-rotation to a permanent value corresponding with the specific rotation of  $\alpha$ - $\beta$  glucose in equilibrium.

More recently studies by Winter and Smith (3) have suggested that there may be an actual difference in the type of sugar which is present in normal and diabetic blood. These investigators called attention to the fact that in comparing the rotatory power of sugar obtained from the blood of normal and diabetic individuals a difference in the specific rotation was observed. They found that the sugar from the blood of normal animals and men, when examined after its separation by a rather lengthy process from the blood protein, showed a rotation of polarized light below that corresponding to the ordinary equilibrated  $\alpha$ - $\beta$  glucose. On standing, the optical rotation rose until in a day or two it became constant and agreed with the rotation that would be expected from the amount of glucose indicated by copper reduction determinations. In diabetic individuals, however, this diminution of optical activity and subsequent rise was not observed. It was suggested that these results might indicate the presence in normal blood not of the more stable varieties in which glucose exists in a simple solution, but of a less stable variety such as that identified by Irvine and his coworkers and styled  $\gamma$  glucose. These experiments were subsequently extended (4) to include the blood of diabetic patients who had been treated with insulin, and from these studies they concluded that in diabetics the decreased amount of blood sugar caused by the injection of insulin contained a greater proportion of normal blood sugar than that of the untreated diabetic.

Another interesting experiment has also been performed by these same workers (5) who have reported that when solutions of glucose and fructose are incubated in vitro at 37° in phosphate buffer solutions together with small amounts of insulin and liver extract, their

rotations were altered in a levo and dextro direction respectively, whereas the copper reducing power remained unaltered

Recently, however, most of this work has failed to receive confirmation in the hands of other investigators. The experiments of Hewitt and Pryde have been challenged by Stiven and Reid (6), who have repeated their work and have been unable to confirm the former's results. Similarly van Creveld (7) has obtained negative results and also Hume and Denis (8) who report a series of 21 similar experiments in which they noted in 12 experiments no change in the optical activity of glucose which had been brought into contact with the intestinal mucosa of the rabbit, a small upward rotation in 5, and a somewhat greater downward rotation in 4, showing that unmistakable evidences of the existence of polarimetric changes were present in a large percentage of their experiments, but that these changes did not seem to follow any definite trend.

Doubt has also been cast upon the conclusions of Winter and Smith by Hewitt (9) and by Eadie (10) who repeated their experiments upon rabbits. Eadie is also quoted by Macleod (11) as having shown that in extracts of normal blood polarimetric readings are often obtained which are less dextrorotatory than they should be (as judged from their reducing power) and which slowly became greater on standing, but this instead of indicating the existence of  $\gamma$  glucose, might have depended on the presence either of glucosides which gradually became hydrolyzed on standing, or of traces of other levorotatory substances which gradually became destroyed. It is also pointed out that the results of Winter and Smith rest upon polarimetric readings which were extremely small in magnitude, and furthermore, that the change in optical activity required a time interval amounting to several days which would not be expected if this were due to a highly reactive type of sugar.

Another investigator who has attacked the same problem and who has in some measure repeated Winter and Smith's work is van Creveld (7). He eventually abandoned the lengthy methods of deproteinization of blood as advocated by Winter and Smith, choosing instead to work with the aqueous humor of the eye, serum ultra-filtrates and artificially produced transudates. With the aqueous humor

he noted that reduction and optical rotation determinations showed close agreement and mutarotation could not be detected. With the serum ultrafiltrates and transudates no changes were noted in optical rotation, but there always was a small difference between optical rotation and reduction in favor of the former.

Visscher (12) has also repeated Winter and Smith's experiments and reports that the supposed differences between normal and diabetic blood which they have noted could be produced by varying the H ion concentration of the blood filtrate. If the filtrate was nearly neutral it resembled normal blood, if strongly acid diabetic blood. He also suspected that optically active substances other than dextrose, which were not eliminated by the deproteinization of the blood, might play an important rôle in the observation. Quite recently Denis and Hume (13) in a careful and broad repetition of Winter and Smith's work have likewise failed to corroborate the latter's work.

Apparently, therefore, the balance of evidence obtained by the more recent investigators, who have been quoted above, seems to indicate that this is a rather sterile method of attack in our efforts to investigate dextrose metabolism. The field has not, however, been exhausted. Clark's original work does not seem to have been repeated and the proof or disproof of his theory is evidently of fundamental importance in our conception of the manner in which glucose may possibly be influenced within the body in preparation for its utilization.

With the thought that the urine might contain enzymes or other factors which are present in the blood of normal and diabetic individuals, it seemed interesting to study its effect upon added sugar by a series of polarimetric observations. This is evidently not quite comparable to a study of the actual difference between the sugar physiologically present in blood and that seen in diabetics. However, a comparison of the sugar in normal urine and that found in the diabetic is difficult because of the rather complex nature of the former and its exceedingly small quantity.

In the course of this work the problem divided itself naturally into a number of different phases, the original primary object of this study was the effect which normal urine might have upon added glucose as compared with diabetic urine upon the glucose naturally present

Secondarily, a further comparison was drawn between the effect of both normal and diabetic urine upon added glucose. The incidental problem, which proved to be of prime importance was that of a comparative study of the optically active substances in normal and diabetic urine and their tendency to change on standing.

Our experiments are in some measure comparable to those of Hewitt and deSouza (14). These investigators, working on the basis that the sugar of the blood could be best removed through the physiological action of the kidneys, studied the optical and reducing properties of various sugars which were excreted in the urine of experimental animals after their intravenous injection. As a result of their experiments they concluded that, following the intravenous injection of equilibrated solutions of  $\alpha$ -glucose,  $\alpha$ -fructose and  $\alpha$ -galactose into rabbits and dogs, no stereo-chemical changes were noted and the equilibrium of the sugars was unaltered in the excreted urine. They further emphasized the fact that polarimetric estimation of reducing sugar in the urine may give fallacious results unless controlled by other methods.

#### METHODS

For the estimation of glucose by copper reduction, Benedict's quantitative method was employed (15). In using this method it was found necessary to adhere strictly to certain points of technique in order to secure uniform results and, although the procedure is well known, the exact technique as employed in these experiments is given. It was as follows: Twenty-five cc. of Benedict's copper sulphate solution were put into a small wide mouthed flask, together with 7 gm. of anhydrous  $\text{Na}_2\text{CO}_3$ . This solution was boiled over a low flame for exactly 5 minutes and then 3 cc. of distilled water were added. The solution of which the glucose content was to be determined was then added drop by drop from a 10 cc. burette graduated in twentieths of a cubic centimeter. In the case of urine, when the titration was about two thirds finished a drop of octyl alcohol was added to prevent excessive foaming. As the end point was approached a time interval of 3 seconds was allowed between each drop to promote complete reduction. In the event that less than 4 cc. of glucose solution were required to complete the reduction, the solution



was diluted accordingly. In all instances the determinations were run in duplicate or triplicate. Using this method on various glucose solutions which were generally of a concentration of about 1 per cent, the average error from a series of 10 determinations was calculated to be 0.5 per cent, the maximum being 0.8 per cent. This degree of accuracy compared favorably with that obtained by the Folin-McElroy method (16) which showed a slightly greater error in our hands. Benedict's method proved also to be more advantageous for these experiments in that it was the simpler of the two.

For the standardization of the reducing method a series of sugar determinations were run upon known solutions of glucose, the value of which had been determined polarimetrically. For the specific rotation of glucose  $+52.8^\circ$  was adopted.

The polarimetric determinations were made with a Reichert instrument which was graduated to read in hundredths of a degree. The readings were made in a 189.4 mm tube using a 100 watt Mazda lamp and an appropriate dichromate solution filter for the light source. Final determinations represented the average of 5 successive readings not varying over  $0.03^\circ$ . This gave results which could be compared with a fair degree of accuracy to the third decimal place.

The urine employed was obtained freshly passed from normal individuals and from patients with apparently uncomplicated diabetes mellitus. Only those specimens of urine were chosen which did not contain acetone or diacetic acid and which failed to show appreciable quantities of albumen by the routine clinical tests. For the added sugar Merck's dextrose was used in all of the experiments.

A freshly prepared 5 per cent solution of glucose was made up for each experiment. This was allowed to boil for ten minutes to obviate mutarotatory phenomena, and was then cooled and made up to its original volume.

From the freshly voided specimens of normal and diabetic urine 25 cc samples were transferred into 100 cc volumetric flasks, 20 cc of the 5 per cent glucose solution were added and the whole diluted to the mark, making a final concentration of 1 per cent glucose. At the same time 25 cc samples of the same urine specimens were diluted with water to a volume of 100 cc without the addition of glucose. A 1 per cent solution of glucose in water was also made to serve as

a control for each experiment. The solutions were then stoppered and placed in the water bath at  $38^{\circ}$  and as soon as possible polariscopic readings were commenced. These were continued on the diluted samples of urine, the diluted urine plus glucose and the control at one half to one hour intervals for a period of five hours. At the beginning and end of this time copper reducing determinations were run on each of the solutions containing glucose. At the end of 5 hours one drop of toluol was added to each specimen and the solution placed on ice. On the following day a single polariscopic reading and copper reducing determination was made.

Owing to the difficulties of obtaining strictly sterile urine the experiments were not carried out under absolute aseptic technique. The glassware containers were sterilized and ordinary precautions to avoid contamination were utilized. In a few instances bacterial growth became apparent in the urine during the initial 5 hours which was invariably evidenced by the fact that the urine became cloudy, and at the same time both polariscopic and copper reduction values began to show a parallel fall. Specimens showing such evidences of contamination were always discarded.

### RESULTS

One representative experiment has been charted in graphic form (fig 1) in order to illustrate the manner in which the results have been recorded and subsequently studied. From this chart it will be noted that the curves at the bottom of the figure designate a series of polariscopic readings upon plain urine, Nos 1 A and 2 A representing normal specimens which remain levorotatory throughout the course of the experiment and No 3 A a diabetic specimen which is dextrorotatory for the first 6 hours followed by a sharp drop below the zero mark on the following day. The three curves in the upper half of the figure, Nos 1, 2 and 3 designate a corresponding series of polariscopic readings made simultaneously upon the same urine specimens to which 1 per cent glucose solution had been added, and a fourth curve, No 4, represents similar readings upon a control solution of 1 per cent glucose.

Curves 1 and 2 of normal urine-glucose solution start with relatively high polariscopic readings which diminish slightly during the first

6 hours Curve 3 the diabetic urine-glucose solution starts relatively low and climbs upward during the first six hours to be followed by a sharp drop on the following day The control solution of 1 per cent glucose is represented by curve No 4 which roughly maintains a

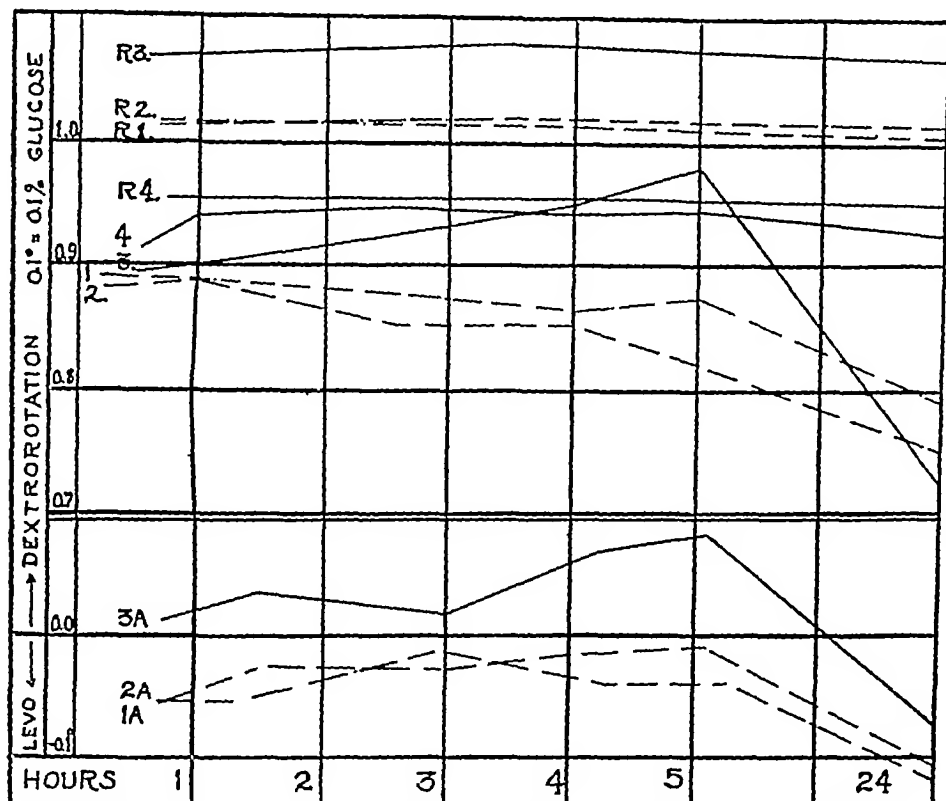


FIG 1 TYPICAL EXPERIMENT SHOWN IN GRAPHIC FORM

Lines 1 A and 2 A represent polariscopic readings upon normal urine, 3 A diabetic urine Lines 1 and 2 represent polariscopic readings upon normal urine to which 1% glucose has been added and line 3 diabetic urine similarly treated Line 4 a control solution of 1% glucose Lines R 1, 2, 3, and 4, represent corresponding reducing values of the solutions containing glucose

straight line throughout the experiment One cannot but notice the distinct influence which curves 1 A, 2 A and 3 A seem to exert upon 1, 2 and 3 They can hardly be said in this instance to show definite parallelism but the major fluctuations noted in the curves 1, 2 and 3 show a counterpart in curves 1 A, 2 A, and 3 A, particularly the latter.

At the top of the figure Curves R 1, R 2 and R 3 designate the series of values obtained from reducing determinations made upon the urine-glucose solutions. It will be noted that the reducing value curves adhere quite closely to a straight line throughout the experiment and that they are considerably higher in the case of the urine-glucose solutions than the values obtained by estimating the glucose content polariscopically. In the case of the control, however, curve R 4 the polariscopic and reducing values approach each other quite closely.

The further results of the series of experiments are shown graphically by composite curves. The changes encountered in the optical activity of normal urine alone are given in figure 2 and in tabular

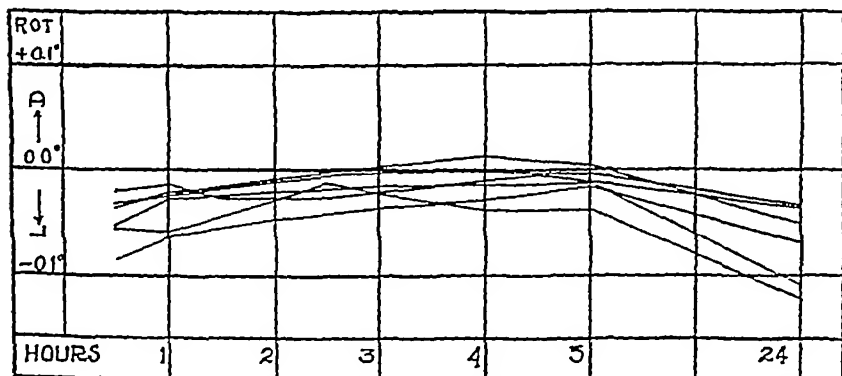


FIG 2 ASSEMBLED CURVES OF POLARISCOPIC READINGS UPON NORMAL URINES

form by table 1. The results of a series of seven experiments are given in which readings were made at hourly and half hourly intervals for a period of 5 hours followed by a final reading at the end of 24 hours. It will be noted that appreciable changes occur during this period of time and, that in the 7 experiments shown, a fairly uniform trend is followed. In all instances the initial reading of the normal urine samples proved to be levorotatory, varying in degree from  $-0.020^{\circ}$  to  $-0.085^{\circ}$ . On standing at body temperature a gradual diminution in the levorotation invariably occurred so that in the course of 3-4 hours the reading in all instances approached the zero point and in one instance (number 7) it became dextrorotatory. The final reading taken at the end of 24 hours after the specimens had been

TABLE 1  
*Normal urine*

Number	½ hour	1 hour	1½ hours	2 hours	2½ hours	3 hours	3½ hours	4 hours	4½ hours	5 hours	24 hours
	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>
1	-0 067	-0 050			-0 045	-0 038		-0 015		-0 006	-0 064
2	-0 085	-0 063								-0 028	-0 070
3	-0 053	-0 058			-0 013			-0 039		-0 039	-0 121
4	-0 055	-0 025			-0 028			-0 011		-0 010	-0 111
5	-0 034	-0 028	-0 015		-0 002		-0 002		-0 008		-0 040
6	-0 020	-0 013	-0 028		-0 016		-0 018	+0 012	0 005		-0 038
7	-0 032	-0 025		0 027	+0 002					-0 003	-0 051

kept on ice invariably showed a shift back towards the original reading, and in two instances the final determination was negatively greater than the original

In the same manner the changes encountered in the optical activity of diabetic urine alone are shown in figure 3 and table 2. The initial readings in this series all proved to be dextrorotatory although the amount naturally varied far more than in the normal samples, being from  $+0.008^\circ$  to  $+0.162^\circ$ . It will be noted that successive readings in this series showed a rather irregular picture. Fairly wide fluctuations were observed and in two instances a well defined rise was noted at the end of 3 hours followed by a subsequent fall

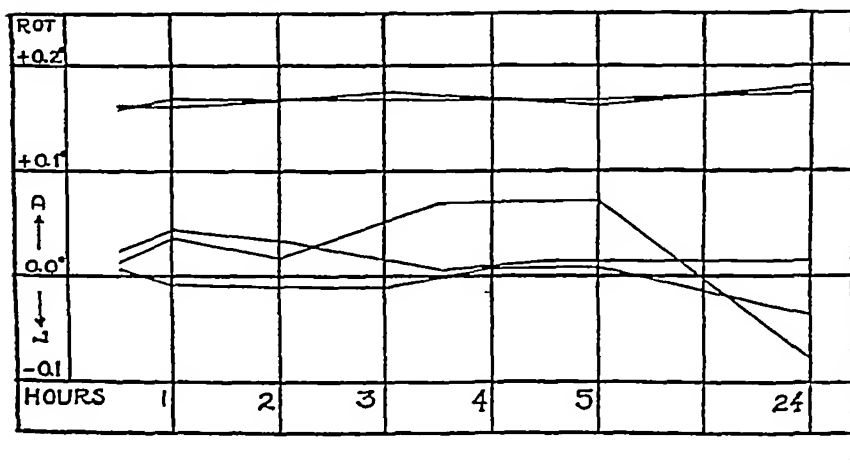


FIG 3 ASSEMBLED CURVES OF POLARISCOPIC READINGS UPON DIABETIC URINES

at the end of 24 hours well below the zero mark. In general the fluctuations of the other three specimens during the first 5 hours did not show any definite trend, but adhere more or less to a straight line.

In comparing the assembled curves in the case of both normal and diabetic urine one is impressed with the tendency for the readings to shift above and below the zero point. This might be attributed to some optically active substance shifting from a dextrorotatory to a levorotatory character or vice versa. It is, however, more probable that the readings represent the total effect of several optically active substances presumably including small quantities of sugar and of

TABLE 2

*Diabetic urine*

Number	$\frac{1}{2}$ hour	1 hour	1½ hours	2 hours	2½ hours	3 hours	3½ hours	4 hours	4½ hours	5 hours	24 hours
	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>	<i>degrees</i>
1	+0 163	+0 160				+0 175				+0 165	+0 180
2 + (I)*	+0 162	+0 173				+0 167				+0 167	+0 182
3 -	+0 013	+0 035		+0 018			+0 007	+0 071		+0 075	+0 075
4	+0 024	+0 045	+0 040	+0 034				+0 011	+0 016		+0 017
5	+0 008	-0 007		-0 009		-0 006			+0 010		-0 033

\* *Note* Nos 1 and 2 are samples of the same specimen To No 2, 10 units of insulin were added It will be noted that there are on appreciable changes as a result of the addition of insulin.

glucuronic acid or glucuronates, some of which are dextrorotatory and others levorotatory in character. Changes in any one of these optically active substances would of course influence the total reading. It is clear that in adding glucose to urine one is adding a substance which is optically active to a solution which already contains optically active substances and the polariscopic reading of the resultant solution will represent the sum total of these. A study, therefore, of the changes in the optical rotation of the added glucose might seem to be largely dependent upon the associated changes usually occurring in urine. In order to estimate, therefore, the degree of rotation of the added sugar, it might seem justifiable to read the urine with and without added sugar at stated intervals, and subtract the readings of the simple urine from that of the urine to which glucose has been added. The values obtained, however, by this method would be valid upon the assumption, of which we have no assurance, that the usual changes in optical activity noted in simple urine actually takes place, once glucose has been added.

On the basis of our 7 experiments, curves have been drawn to represent, upon the assumption just named, the values of the optical rotation of the sugar added to urine. The ordinates in these curves designate the differences between simultaneous readings of the urine with and without added sugar. The results of normal and diabetic samples are shown in figures 4 and 5 respectively.

In the case of the normal samples of urine a gradual apparent diminution of about  $0.05^\circ$  is observed during the first few hours with as a rule a subsequent slight rise.

With the diabetic urines the curves are irregular but without any consistent rise or fall. An explanation of the minor fluctuations is not attempted but when the curves are viewed critically it does not seem that the optical activity of the added sugar has been appreciably influenced by the urine.

It will be finally noted that in all of these experiments the reducing determinations of the sugar in urine remain constant, but as stated before they show values considerably higher than those obtained by polariscopic determinations.



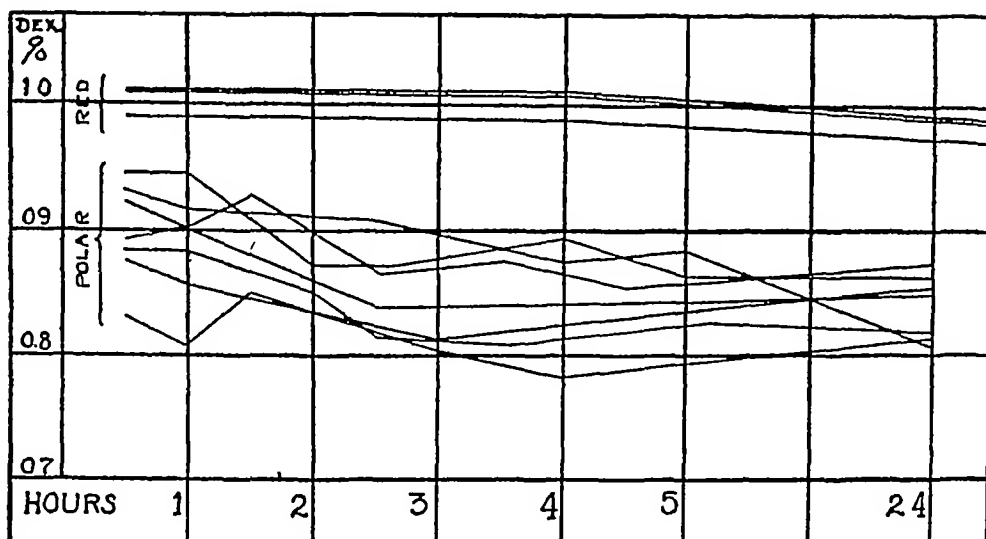


FIG 4 ASSEMBLED CURVES OF THE INCREMENTS IN POLARISCOPIC AND REDUCING VALUES FROM GLUCOSE ADDED TO NORMAL URINE

The lower curves represent only the computed value of the glucose increment as determined polariscopically, the upper curves represent the combined reducing values of urine and the glucose increment

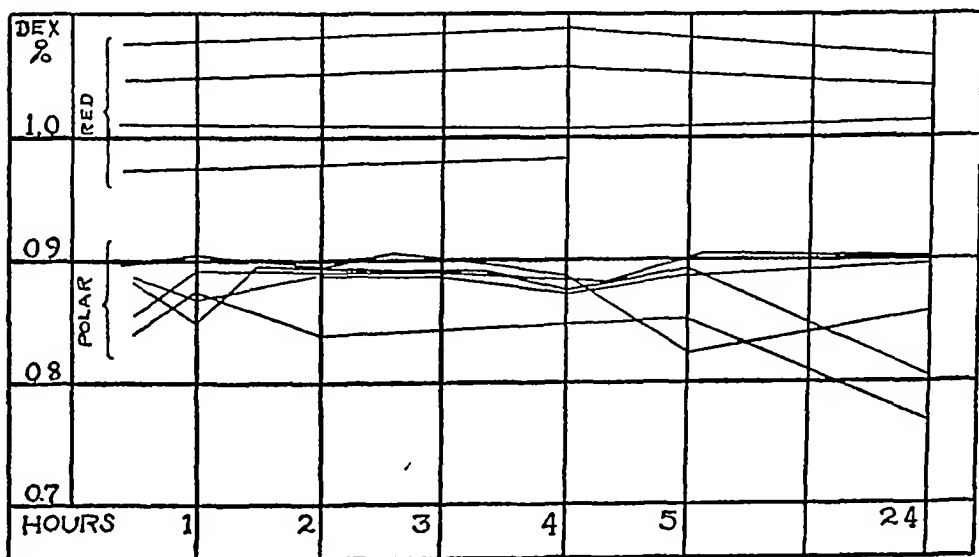


FIG 5 ASSEMBLED CURVES OF THE INCREMENTS IN POLARISCOPIC AND REDUCING VALUES FROM GLUCOSE ADDED TO DIABETIC URINE

The lower curves represent only the computed value of the glucose increment as determined polariscopically, the upper curves represent the combined reducing values of urine and the glucose increment

## SUMMARY

On standing appreciable changes in the optical activity of dilute samples of normal urine occur. These consist in a diminution of the levorotation noted in fresh urine until at the end of 3 to 5 hours the zero point is approached. Subsequently until the end of 24 hours there is an increase of levorotation and return to initial values.

Similar changes are noted in dilute samples of diabetic urine but the general course of these changes seems to be more irregular than in the normal.

The increment in polariscopic readings produced by the addition of 1 per cent glucose to normal urine diminishes slightly during the first few hours with subsequently, up to 24 hours, no further change.

Polariscopic readings produced by 1 per cent glucose added to diabetic urine shows only such variations over a period of 24 hours as could be accounted for by the changes occurring in the optically active substances already present in the urine.

The difference in behaviour of glucose when added to normal and diabetic urine, is, however, quantitatively too slight to permit deductions as its true significance.

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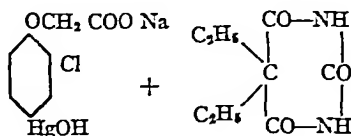
# OBSERVATIONS ON THE USE OF NOVASUROL IN EDEMA DUE TO HEART FAILURE

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Novarsurol is an organic compound containing mercury, which was introduced by the Bayer company as an antisyphilitic agent. Its therapeutic use in this disease was first described by Zeiler (1917) Its constitution is sodium oxymercuro-ortho-chlorphenol-oxylacetate with diethylmalonylurea and may be represented as follows (White, 1924)



It is prepared for use as a 10 per cent neutral sterile solution The marked incidental diuretic action caused by its administration was noted soon after its introduction, especially in those cases in which considerable edema had developed Its use as a diuretic in cases other than those of syphilitic origin was naturally suggested by this occurrence

It has been known for a long time that the administration of mercury produces diuresis Even as early as 1799 Fernar stated that the diuretic action of digitalis was increased when given in conjunction with calomel Jendrassik (1886, 1891) was the first to draw attention to the striking effects that could be produced by the use of calomel alone He administered repeated small doses by mouth and obtained a remarkable diuresis in cases of edema This treatment was in vogue for some time but gradually became much less used as different observers reported deleterious after effects on the kidney There has been a good deal of debate as to the mechanism by which this

diuresis is produced. The majority of observers now, as is seen in Cushny's (1917) review of the literature, favor the view that it acts directly on the kidney.

Novasurol has already been used in many types of edema. It has apparently no effect on inflammatory exudates. Practically all observers are agreed that it is contra-indicated in renal disease. In the ascites due to cirrhosis of the liver and carcinoma, reports of the results obtained vary considerably. In heart disease on the other hand remarkable results have been described by several observers. The first account of its use as a diuretic was given by Saxl and Heilig (1920). They obtained diuresis up to three liters by giving 1 to 2 cc. by intramuscular injection, and the patients lost weight corresponding to the diuresis. The chloride in the urine was increased both in total quantity and in concentration. The injection of atropine inhibits the diuresis. In a small number of their cases troublesome attacks of diarrhea developed. No evidence of kidney damage occurred. The fact that this drug can remove edema which had resisted the usual forms of medication has been confirmed by several observers. The increase in chloride output has also been confirmed by all who have studied this point. Saxl and Heilig (1920) in addition found that the total quantity of nitrogen excreted in the urine was unaltered. Mühling (1921) states that the total nitrogen, urea and creatinin in the urine were increased. He says this increase may occur one or two days later than the other effects.

The mechanism by which novasurol acts has aroused a considerable amount of controversy. Saxl and Heilig (1922) believe that the action is on the extra renal tissues. They studied the protein content of the plasma by means of the refractometer and found a decrease in the early stages in the majority of cases. Consequently, they think that the diuresis is secondary to hydremia. In their opinion fluid and salt are first transferred to the blood from the tissues and then excreted by the kidney. The chloride in the plasma was first reduced, the lowest value being obtained in 4 to 6 hours, and later began to rise again. In the cases in which diuresis was arrested by giving atropine hydremia and hyperchloremia developed. Saxl and Heilig (1923) made some experiments on dogs to which uranium nitrate had previously been given. When novasurol was injected soon after giving uranium, dilution of the blood took place and an increase in the chloride content, followed some hours later by concentration of the blood and diminution in the plasma chloride. Some time later after uranium nephritis became established there was a dilution of the blood and the chloride content was increased. On giving novasurol now diuresis occurred without further dilution or increase in chloride concentration. In still later stages of uranium poisoning the effect of novasurol was to produce polyuria without any increase in the chloride in the urine. At this stage they think novasurol reactivates uranium.

Nonnenbruch (1921) and Eppinger (1921) confirmed the work of Saxl and Heilig on the changes in the blood and agreed with them in believing that the action of the

drug is on the extra-renal tissues Bohn (1923) as the result of experiments on normal and nephrectomised rabbits also obtained evidence of a primary dilution of the blood and agreed with these authors in considering the action extra renal. Tezner (1923) studied the rate of absorption of normal saline solution injected subcutaneously into the instep in children and found that this was absorbed more rapidly when novasurol had been previously administered. This is additional evidence he thinks that the action of the drug is on the tissues

Mühling (1921) was unable to confirm the changes found in the blood by the observers just mentioned and believed that the action of novasurol is on the kidney tissue. Klucke (1922) is of the same opinion Bleyer (1922) on the other hand found the changes in the blood to be so slight that he was unwilling to draw conclusions from them Brunn (1921) considers that no conclusive evidence has been brought forward as to the site of action, while Fodor (1923) thinks that it acts neither directly on the kidneys nor on the tissues, but on a hypothetical centre in the medulla which regulates water and chloride metabolism.

An extensive study of the toxic effects of novasurol has been made on syphilitic subjects by Zeiler (1917), who administered 5000 injections to 900 patients He found that there was vomiting in 2.4 per cent of cases and 0.5 per cent of injections, diarrhoea in 6 per cent of cases and less than 1 per cent of injections, stomatitis in 4 per cent of cases Fainting occurred in a very small number From these effects recovery was rapid and they were considered to be of little importance. There was kidney irritation in less than 1 per cent of cases and only in those in which evidence of previous kidney disease existed. He states that albuminuria when present progressively improved even when it had been produced by calomel or grey oil The only other toxic symptoms which have been described occurred in a few cases in which hemorrhagic colitis appeared One case of hemorrhagic encephalitis was attributed to the action of the drug All those who have studied the subject clinically agree that toxicity is infrequent and rarely severe and that there is no evidence of kidney damage unless the kidney has previously been diseased

The present investigation has been undertaken to study the effect of novasurol on edema in such cases of cardiac disease as were resistant to treatment with diet, rest, and drugs, and to find whether the drug produced any deleterious effects on the organism The alterations produced in the various constituents of the urine, blood and edema fluid, were also studied with a view to obtaining some light on the mechanism by which the drug acts

#### METHOD OF INVESTIGATION

The patients examined were treated throughout the investigation by rest in bed and salt free diets, except in two cases which received 1

gram of salt per diem Their fluid intake was restricted to 1200 cc per diem Before novasurol was given digitalis was administered until one was assured that no effect on the excretion of urine was obtainable by this means - In four of these patients who were suffering from fibrillation of the auricles, it was thought advisable, in order to study the effect of novasurol under conditions in which as many factors as possible remained constant, to continue the administration of digitalis in amounts sufficient to maintain the ventricles at a constant rate Digitalis was, however, never given during the test itself In two cases of auricular fibrillation and in the cases in which the cardiac rhythm was normal, the heart was not under the influence of digitalis when novasurol was being investigated During the preliminary period of the investigation the urine was collected in 12 hour specimens In estimating the fluid intake both the actual fluid and food fluid were measured The volume of urine, specific gravity and the chloride output were determined in each specimen When the water and chloride output reached a constant level, novasurol was given In many of the observations the urine was collected in 3-hour periods for 24 hours before and 48 hours after the administration In these specimens the volume, specific gravity, chloride, and sometimes the urea and ammonia of the urine were estimated In the interval between injections the volume and chloride of the urine continued to be studied in 12 hour specimens as before Chloride was estimated by a Volhard titration and urea and ammonia by the method of Van Slyke and Cullen (1914) Specimens of blood taken were  $4\frac{1}{2}$  hours after the administration of novasurol and at the corresponding time on the previous and following days Four and one half hours after the drug had been given was selected as the time for obtaining the blood samples as at this time the response to novasurol was well established We estimated urea by the method of Van Slyke and Cullen (1914), and plasma chloride by the method of Van Slyke (1923) The chloride in the plasma and urine were determined in order to study the remarkable changes in salt metabolism reported after novasurol injection Urea estimations in blood and urine were made primarily to discover whether kidney function was damaged by the drug and secondarily to observe in what way, if any, urea excretion was affected In many instances we also studied the corpuscular volume by means of the

hematocrit and the hemoglobin percentage by Haldane's hemoglobinometer In the cases in which we investigated the question of dilution or concentration of the blood, specimens were taken one half hour after the injection and at frequent intervals up to 7 hours In these specimens we determined the percentage of protein in the plasma by means of the refractometer, plasma chloride, corpuscular volume and, in some cases, the hemoglobin percentage When observations were made on the edema fluid this was done by puncturing the tissues with a Southey tube and collecting the fluid which exuded On such occasions the edema fluid was collected at the same time as the sample of blood and the chloride content of the fluid was estimated by the same method as was used for plasma chloride

Novasurol was administered in doses of 1 to 2 cc by intramuscular or intravenous injection In the two cases in which the latter was used there was found to be no advantage in it over the intramuscular method A preliminary injection of 1 cc was given to guard against the possibility of an idiosyncrasy to the drug Afterwards injections of 2 cc were usually employed The drug was injected into the buttock in most instances, but in those in which there was edema of this region it was injected into some edema-free muscular region, usually the muscles of the back. Injections were not repeated at intervals shorter than four days, except in the case of the injection following the preliminary 1 cc, this was given two days later Previous observers found rapid repetition inadvisable

#### OBSERVATIONS

The cases which we have selected for observation were in the most advanced stages of heart failure Clearly a new diuretic has its principal advantage if it is shown that with it edema fluid can be removed from cases in which it had been demonstrated that the usual methods of treatment failed We have administered the drug to eight cases

*Case 1* I K., Hospital No 4836 The patient was a man of 37 years of age On admission he suffered from marked dyspnea and palpitation He was extremely cyanosed The heart was enlarged and systolic and diastolic murmurs were heard at the apex The pulse was rapid and irregular There was fluid in the right pleural cavity and moist râles were heard over both lungs The liver was en-



larged and ascites was present. There was edema of the legs. Digitalis slowed the heart but failed to alter the edema. Diuretin was also unsuccessful. As the result of giving novasurol the patient's clinical condition improved enormously. Dyspnea while in bed disappeared. The cyanosis became much less marked. The peripheral edema, ascites and pleural exudate disappeared and the liver was reduced in size. These tended to recur unless controlled by further injections. The diagnosis was mitral stenosis, auricular fibrillation, acute cardiac decompensation.

*Case 2* S M, Hospital No 4851. The patient was a man of 31 years of age. On admission he was dyspneic and cyanotic. The heart was enlarged and there were systolic and diastolic murmurs to be heard at the apex. The pulse was rapid and irregular. There were moist râles all over the chest but no fluid in the pleural cavities. The liver was enlarged and there was well marked ascites. There was edema of the legs. Digitalis showed the heart but failed to alter the edema. Diuretin also failed to produce any action. Theocin produced a slight diuresis but caused symptoms of gastric irritation. Novasurol injections produced a marked improvement. Dyspnea and cyanosis disappeared. The edema of the legs and the ascites disappeared and the liver was reduced in size. Improvement continued without further injections. The diagnosis was mitral stenosis, auricular fibrillation, acute cardiac decompensation.

*Case 3* F S, Hospital No 4282. The patient was a female aged 20 years. On admission she was dyspneic and complained of marked palpitation. There was considerable cyanosis. The cardiac pulsation was marked so that the left side of the chest heaved with each beat. The heart was enlarged and systolic and diastolic murmurs were heard all over the chest, especially in the region of the apex. The pulse was rapid and irregular. There was no exudate into the pleural cavities but a few moist râles were heard. The lower border of the liver was mid-way between the umbilicus and pubis. There was neither ascites nor edema of the legs but there was slight edema of the sacral region. Digitalis even in small doses occasioned the onset of pulsus bigeminus and made the clinical condition worse. During previous admissions to hospital she had reacted well to digitalis. Theocin was given without effect. After an injection of novasurol the improvement was striking. Dyspnea disappeared. The color improved and palpitation became slight. The heart rate was reduced, pulsation in the neck was absent and in many instances the size of the liver was reduced 5 cm. This improvement continued for some time after each injection and then the patient would become suddenly worse. Eventually a stage was reached when no response was elicited and the patient died. The diagnosis was mitral stenosis, auricular fibrillation, acute cardiac decompensation.

*Case 4* M, D, Hospital No 4731. The patient was a colored man aged 48 years. He suffered from severe dyspnea. There was cyanosis of the mucous mem-

branes The heart was large and there were systolic and diastolic murmurs to be heard at the aortic area The pulse was slow, irregular and water-hammer There was a considerable amount of fluid in the right pleural cavity There was slight enlargement of the liver and fluid in the abdominal cavity There was no edema of the legs Thoracocentesis was performed on three occasions on account of respiratory distress The patient was thoroughly digitalized There was marked improvement in the clinical condition, but fluid persisted in the abdomen As the result of novasurol injections this was completely removed and has not reaccumulated The diagnosis was aortic disease, auricular fibrillation, acute cardiac decompensation

*Case 5* S N, Hospital No 4971 The patient was a man aged 38 years He was nervous and suffered from dyspnea and cyanosis The pulse was regular The systolic blood pressure was 152, the diastolic 120 The heart was enlarged but there were no murmurs to be heard There was fluid in the right pleural cavity and moist râles in the lungs The liver was slightly enlarged and ascites was present There was edema of the legs There was no evidence of kidney insufficiency After giving digitalis diuresis began but this was not maintained sufficiently long to produce a marked reduction in the edema. The clinical condition improved while the diuresis lasted but then a relapse took place. The patient did not react well to the first dose of novasurol and no improvement occurred The second injection had a better effect and was followed by definite improvement The patient died suddenly at stool five days after the last injection had been given His clinical condition appeared better on the day he died than it had been since admission The diagnosis was essential hypertension, cardiac hypertrophy, acute cardiac decompensation

*Case 6* L L, Hospital No 4935 The patient was a female aged 46 years She showed the typical symptoms of exophthalmic goitre She suffered from marked dyspnea and complained of a considerable amount of pain and tenderness over the precordium and left arm Cyanosis was not marked The pulse was slow and irregular The heart was enlarged and a systolic murmur was present at the apex The sounds were clear at the other areas There was fluid in the right pleural cavity and ascites was also present The liver was not enlarged There was edema of the sacral region and legs No improvement followed the use of digitalis Giving theocin resulted in a slight diuresis but was accompanied by symptoms of gastric irritation An hour after 1 cc. of novasurol was given the patient had a rigor lasting a few minutes and the temperature rose to 102° A little later she vomited, she was uncomfortable for several hours but quickly recovered A definite diuresis occurred and next day the patient felt better than before the injection No further injections were given The diagnosis was exophthalmic goitre, auricular fibrillation, cardiac decompensation

*Case 7* C R, Hospital No 4267 The patient was a male aged 55 years. He suffered from marked dyspnea and cyanosis. The pulse was moderately rapid and totally irregular. The heart was enlarged. No murmurs were heard. There were sibilant rhonchi in the lungs but no moist râles were heard. The liver was enlarged and there was well marked ascites. There was considerable edema of the legs and sacral region. The patient showed progressive improvement under digitalis. Novasurol was administered during this period but there was no evidence that any part of the subsequent improvement was due to it. The diagnosis was chronic myocarditis, auricular fibrillation, acute cardiac decompensation.

*Case 8* A B, Hospital No 4919 The patient was a woman aged 44 years. The outstanding symptom was tremendous abdominal distention due to ascites. This had necessitated tapping every three weeks since its onset one year and eight months ago. The patient suffered from dyspnea on exertion and this became marked when ascites was extreme. Cyanosis was slight but increased in proportion to the abdominal distention. Slight precordial pain was present when the abdomen was tense. The pulse was regular. The heart was greatly enlarged and systolic and diastolic murmurs were heard at the apex. The lungs showed no abnormality. The liver was enlarged. There was considerable edema of the legs and sacral region. No benefit followed the use of digitalis. Ascites progressively increased and relief was obtained only by abdominal puncture. Immediately before tapping novasurol was given with some increase in urine output. A second injection produced practically no effect. There was no alteration in the clinical condition. The day after the second injection 13 liters were removed by abdominal puncture. Novasurol injections were not repeated. The diagnosis was mitral stenosis, cardiac decompensation, perihepatitis.

*Summary* These then were cases of severe heart failure in which edema was a prominent symptom. In six cases satisfactory diuresis followed the injection of novasurol. In Case 7 there was no effect from the administration, this case exhibited an increased urine volume following digitalis administration and novasurol was given to see whether it could produce an increase in this diuresis. This did not take place. In Case 8 the effect was very slight.

## RESULTS

### *Changes in the body weight*

In every case in which marked diuresis occurred there was a corresponding fall in weight (table 1 and fig 1). The fall sometimes continued for a few days after the administration. The greatest

fall in weight on the day immediately following an injection was 3 kilograms. Usually it was 1 to 2 kilograms. In the interval between injections there was often a slight gain in weight.

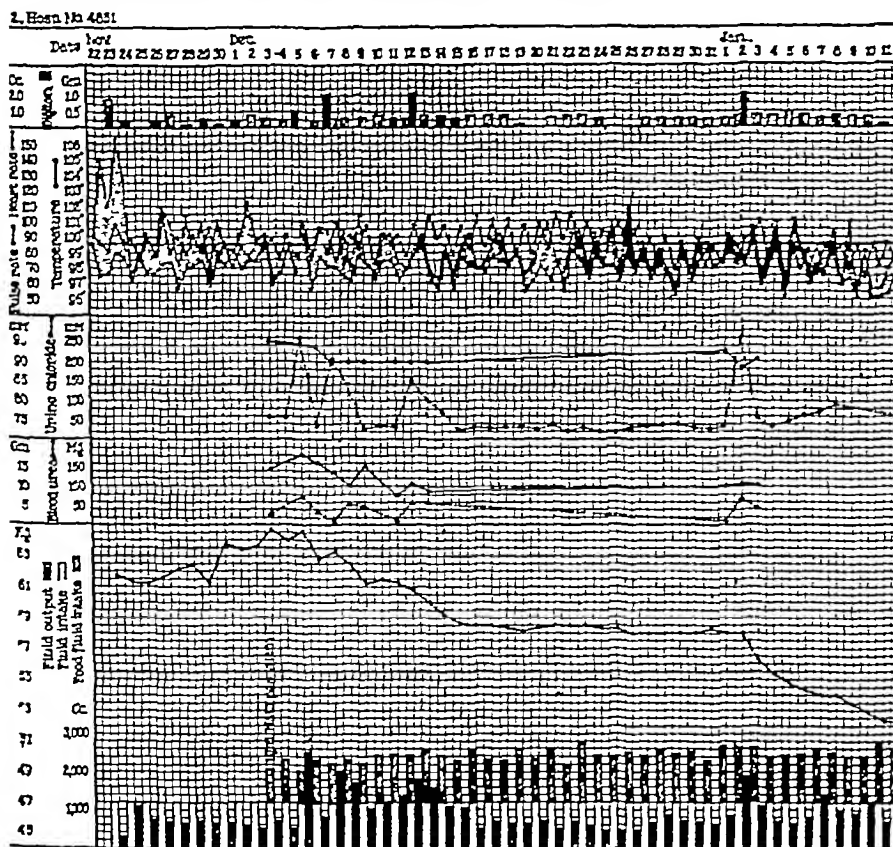


FIG 1 THE EFFECT OF NOVASUROL ADMINISTRATION THROUGHOUT THE COURSE OF TREATMENT (CASE 2)

### *Changes in the urine*

**Volume** The volume of urine excreted varied, but in all cases it increased after giving novasurol to a point above the previous level (table 1 and fig 1). The only exception was Case 7, in which diuresis was already in progress as the result of giving digitalis. Before the administration of the drug the urinary output was usually very small,

being about 300 to 400 cc. per diem. After the administration, in many cases this was remarkably increased. The degree of diuresis which resulted varied with the dose. The maximum effect following the injection of 1 cc. was 1884 cc., but the usual result was from 1000 to 1500 cc. After 2 cc. the maximum volume was 3316 cc. As a rule,

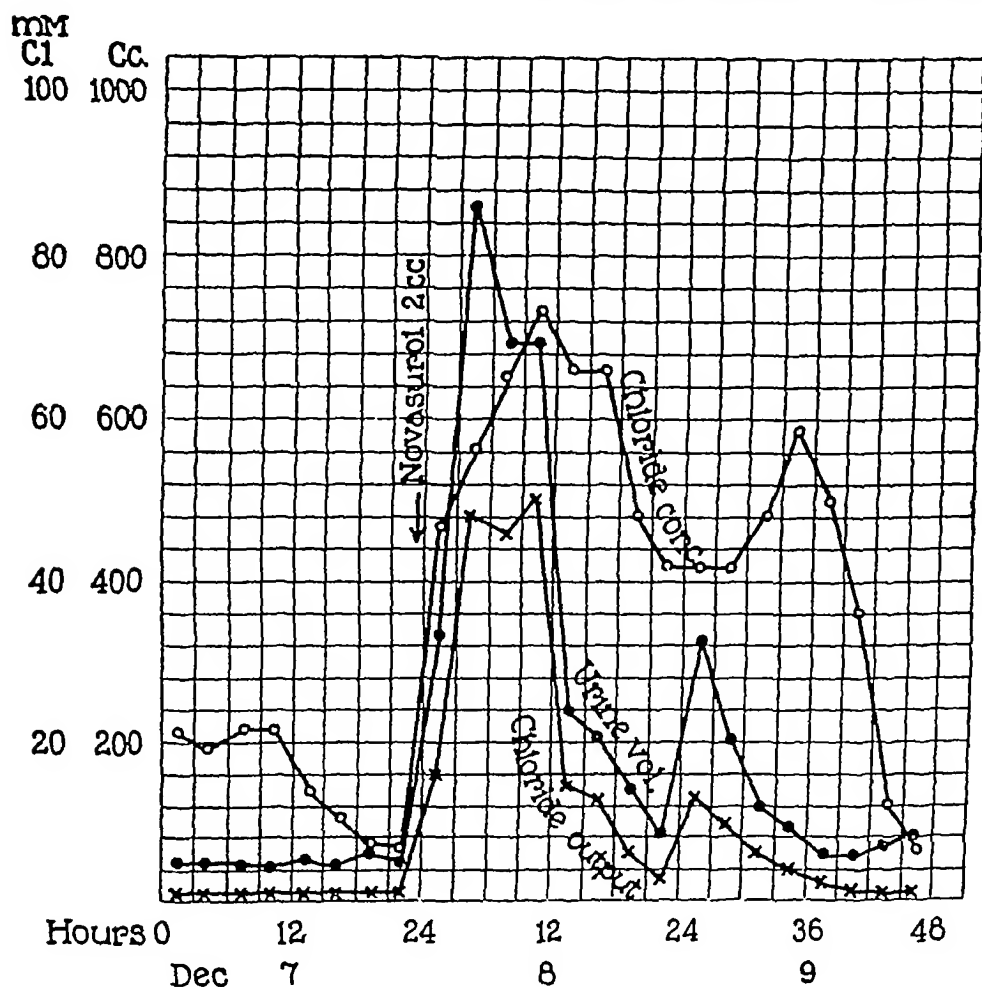


FIG 2 THE URINE VOLUME, TOTAL CHLORIDE EXCRETION AND CHLORIDE CONCENTRATION FOR 24 HOURS BEFORE AND 48 HOURS AFTER NOVASUROL

the output varied from 1500 to 3000 cc. In three cases, although there were definite increases in amount, a reaction as satisfactory as in the other cases did not take place.

Diuresis usually began within the first three hours after the injection of the drug and rapidly attained a maximum in 6 to 9 hours. In cases

where the circulation was more sluggish, the onset of diuresis appeared to be delayed. After attaining a maximum, it gradually fell again until it regained its previous level. This was reached in most cases within 24 hours. In a few cases, however, the augmentation was maintained to a slight extent for another day. There was practically no difference in the rapidity of action between intravenous and intramuscular administration.

*Chloride* One of the most striking effects of novasurol was the remarkable increase of chloride in the urine (table 1). Not only was there an absolute increase in the amount excreted, but the concentration in the urine increased as well (fig 2). The extent of the increase is in fact much greater than that described by Cushny (1917) as following the administration of other diuretics. Before the injection of the drug the chloride output was very small, being less than 50 millimols per diem in almost every case and in many instances, being much less than this. As a result of giving novasurol, the maximum output in 24 hours which was observed was 314.65 millimols. Usually the chloride excretion increased to about 200 millimols per diem. The chloride excretion followed closely the curve of water excretion as to time, except that on the day after injection a slight increase of chloride was more frequently maintained than an increase in water. Two days after the injection the chloride output had invariably fallen to its previous level, and in many cases to a lower level.

*Urea and ammonia* The excretion of urea and ammonia was studied in the 3 hour specimens of urine corresponding to the blood specimens. In three instances urea and ammonia were estimated in 3 hour specimens throughout the 24 hours preceding and the 48 hours following the administration of novasurol. No constant alteration has been observed in the amounts in the urine (table 1). During diuresis the concentration of urea was always considerably reduced but the total output varied. We observed that in some cases the output of urea was greater on the day following than on the day of the injection. The ammonia in the urine showed no constant changes. It varied in the same patient on different occasions. The changes in these constituents were so small that it is evident that the drug has little action on their excretion.

*Abnormal urinary changes* In all cases observations have been

TABLE 1  
*Results of novasurol administration*

CASE	DISEASE	DATE	DOSE	WEIGHT kilos	VOLUME OF URINE* cc	CHLO- RIDE IN URINE* mM	UREA NITROGEN IN URINE† gm	CHLO- RIDE IN PLASMA mM	CHLO- RIDE IN EDEMA FLUID mM	BLOOD UREA N mg per 100 cc	REMARKS
1	Mitral ste- nosis, auric- ular fibril- lation	11-18-23		56 4	310						
		11-19-23	1	56 5	1497						
		11-20-23		55 3	436						
		11-23-23		57 1	296						
		11-24-23	2	56 4	3046						
		11-25-23		54 1	830						
		12-3-23		54 4	340	12 01	2 32	91 07		14 5	
		12-4-23	2	54 5	3316	281 51	10 34	91 55	106 76	15 6	
		12-5-23		51 2	588	31 59	2 09	89 86	105 0	13 8	
		12-7-23		51 3	399	5 41	4 19	87 7	101 2	12 8	
		12-8-23	2	50 1	3247	201 91	7 62	88 6	101 2	10 1	
		12-9-23		48 2	1041	40 74	3 79	86 9		16 7	
		1-5-24		51 4	411	2 35					
		1-6-24	2	51 3	1227	112 10					
		1-7-24		50 3	540	34 14				17 9	
		1-21-24		51 8	444	1 41	1 97	89 0		11 4	
		1-22-24	2	51 6	1204	121 99	1 18	90 5		9 8	
		1-23-24		51 1	617	49 82	0 59	87 5		10 9	

											Urea 30 gms. per diem	
2	Mitral stenosis, auricular fibrillation	2-18-24	2	52 3	988	15 44	16 94	98 1	36 85			
		2-19-24		52 3	3232	314 65	12 38	95 6	32 0			
		2-20-24		50 2	853	14 75	6 99	90 7	28 0			
		2-26-24	1	51 6	812		11 10	88 5	22 8			
		2-27-24		52 2	1219		10 60	90 0	25 6			
		2-28-24		52 3	803							
		12-1-23		64 0	632	60 82	3 37	95 3	14 1			
		12-5-23	1	64 6	2331	253 78	6 82	93 7	17 9			
		12-6-23		62 9	645	27 64	3 48	94 0	15 3			
		12-7-23	2	63 4	2699	288 16	1 92	90 6	105 1			
		12-8-23		62 5	928	62 90	5 80	90 4	105 4			
		12-11-23		61 3	1231	42 25	2 71	90 0	8 7			
		12-12-23	2	60 9	2111	238 01	5 95	90 3	10 8			
		12-13-23		60 0	1108	67 81	5 90	90 3	9 0			
		1-1-24		58 1	715	41 49	1 64	93 9	10 5			
		1-2-24	2	58 0	2411	262 66	6 73	87 9	10 7			
		1-3-24		56 3	994	59 13	1 29	91 3	10 1			
		2-13-24		54 1	696	64 98	5 63	93 8	10 6			
		2-14-24	1	54 1	1138	138 56	3 82	92 8	10 5			
		2-15-24		53 5	471	1 88	3 88	90 6	13 3			



TABLE 1—Continued

CASE	DISEASE	DATE	DOSE	WEIGHT	VOLUME OF URINE*	CHLO- RIDE IN URINE*	UREA NITROGEN IN URINE†	CHLO RIDE IN PLASMA	CHLO- RIDE IN EDEMA FLUID	BLOOD UREA N	REMARKS
			cc	kilos	cc	mM	gm	mM	mM	mg. per 100 cc	
3	Mitral ste- nosis, aortic- ular fibril- lation	2-3-24		42 5	253	8 49		91 0		20 0	
		2-4-24	1	42 6	1884	240 86	4 38	92 0		25 2	
		2-5-24		41 0	608	37 23	6 43	89 0		25 5	
		2-13-24		41 4	453	17 42	3 24 4 59†	94 7		15 3	
		2-14-24	1	41 8	1481	203 28	3 30 4 33	94 3		17 9	
		2-15-24		40 8	487	29 08	4 10 4 15	89 7		14 3	
		2-22-24		41 3	736						Very ill
		2-23-24	2	41 4	3029	342 26					Urea 30 gm. per diem
		2-24-24		39 0	1552	86 02					
		3-10-24		38 6	470	13 78	9 05			28 1	
		3-11-24	2	38 8	2391	303 52	5 95			15 1	
		3-12-24		37 0	417	20 75	4 28			13 1	
		3-19-24		38 2	394	20 45					Urea 30 gms per diem
		3-20-24	1	38 5	1204	159 8					
		3-21-24		38 2	613	63 90				10 6	
		4-3-24		37 2	866	49 68	14 39				Urea 30 gms per diem
		4-4-24	1	37 8	2946	373 0	13 52				
		4-5-24		35 5	1117	43 92					

[illegible]

TABLE 1—Continued

CASE	DISEASE	DATE	DOSE	WEIGHT	VOLUME OF URINE*	CHLO- RIDE IN URINE*	UREA NITROGEN IN URINE†	CHLO- RIDE IN PLASMA	CHLO- RIDE IN EDEMA FLUID	BLOOD UREA N	REMARKS
7	Chronic myo- carditis, Auricular fibrillation	5-1-24	2	kilos	cc	mM	gm	mM	mM	mg per 100 cc	Reacting to digitalis
		5-2-24		84 2	2326	208 08	20 99	91 5		7 6	
		5-3-24		81 7	2158	246 97	23 28	89 8		7 5	
8	Mitral ste- nosis, Chronic myocarditis, Perihepatitis	2-7-24	2	72 9	489	57 14	2 31	95 8		12 7	
		2-8-24		74 2	1240	166 51	2 50	89 3		11 1	
		2-9-24		73 6	283	16 29	1 08	88 3		16 1	
		2-10-24	2	74 1	202	4 81	1 55			17 1	
		2-11-24		75 2	546	49 16	2 0			21 4	
		2-12-24		75 3	146	6 04					

\* Expressed as total for 24 hours

† Urea N was estimated in 3 hour specimens taken during the period 3-6 hours after injection and the 24 hour excretion rate calculated from this

‡ Urea N actually excreted in 24 hours

made daily to see whether there was any evidence of kidney damage as shown by the presence of albumin, casts or red blood cells in the urine. This evidence was completely absent during the period of the investigation which in most cases extended over some months. When these signs were absent previous to the injection they did not appear subsequently. When they were present before they always tended to disappear so indicating improvement.

### *Changes in the blood*

*Plasma chloride* The chloride content of the plasma has been determined in each case at a period of  $4\frac{1}{2}$  hours after the administration of the drug. In most cases there was a slight fall at this period (table 1). On the day following the injection it was invariably lower than before injection. In the interval between injections there was usually a rise towards the original level.

Three observations have been made in which specimens were taken frequently throughout the day of the injection (tables 2 and 3, fig. 3). Two of these were made on patients, and one on a dog. In the two observations on patients a steady fall took place in the plasma chloride after novasurol was injected and this attained its maximum within  $3\frac{1}{2}$  hours, thereafter there was a slow rise. In the dog there was on the contrary a preliminary rise which reached its height  $1\frac{1}{2}$  hours after injection, then a fall which attained its maximum at  $4\frac{1}{2}$  hours and finally a subsequent rise.

*Urea* The urea in the blood also varied considerably (table 1). In some cases it was slightly higher and in others slightly lower during the day of injection. The next day in the majority of cases, however, it was lower than before injection. The blood urea appears to be influenced only in so far as the drug affects the efficiency of the circulation.

*Protein content of the plasma* We have made observations in three instances throughout the day of injection on the amount of protein in the blood plasma by means of frequent refractometer readings. The observations extended from half an hour to seven hours after the injection. The first effect was found to be a fall in the concentration of protein, occurring within an hour and a half after the injection (tables 2 and 3, fig. 3). In one case it reached its maximum in half an hour,

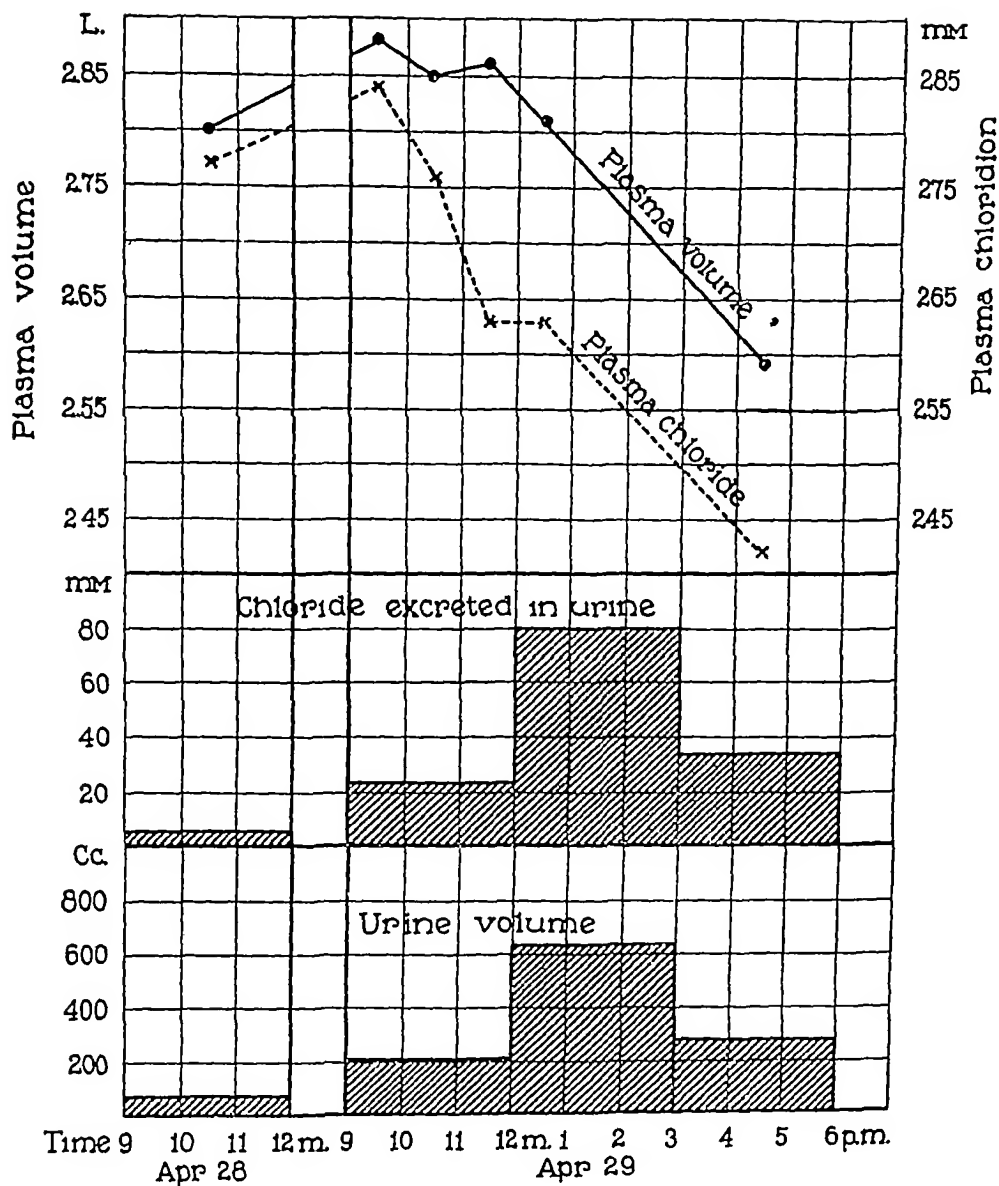


FIG 3 THE CHANGE IN PLASMA VOLUME, PLASMA CHLORIDE, CHLORIDE EXCRETION AND URINE VOLUME AFTER NOVASUROL  
For explanation see text

in another in an hour and a half, and in another in about two and a half hours. There was greater diuresis on the occasion when the lessened concentration took place early. After about two and half hours it began to rise again and continued to do so until in the later stages the blood became much more concentrated than it had been previous to the injection.

*Corpuscular volume* In most of the observations the volume of the cells in the blood was estimated  $4\frac{1}{2}$  hours after the administration of the drug and in every case the blood was found to be slightly more concentrated than it had been previously. In one case in which refractometer readings were made at irregular short intervals throughout the day the corpuscular volume was studied at the same time and was found to follow the same curve as the refractometer readings (table 2).

*Hemoglobin percentage* Readings of the percentage of hemoglobin were taken in most instances  $4\frac{1}{2}$  hours after injection and in every case a slight increase was found. In two cases readings were made throughout the day and in both a primary lowering of the percentage was followed by an increase above the level before the drug was given (table 2). In one case the protein, corpuscles and chloride were also estimated (table 2).

### *Changes in edema fluid*

*Chloride* The chloride in the edema fluid  $4\frac{1}{2}$  hours after novasurol administration was altered in the same direction as that of the plasma, though the changes observed were slightly less marked. A continuous slight fall always occurred and this was maintained in the interval between the injections in contrast to its behavior in the plasma.

### *Evidences of toxicity*

In only one case, a woman, have we had any evidence of an idiosyncrasy to the drug. This was the case of exophthalmic goiter referred to in the protocols (Case 6). She suffered shortly after the injection from rigor, rise of temperature and vomiting, but recovered rapidly from these symptoms. In a few cases there was been complaint of slight headache on the day the injection was given, and sometimes a slight rise in temperature that evening, both being absent the next day.

TABLE 2

*Effect of novasurol injection on plasma protein, plasma chloride, corpuscular volume and hemoglobin percentage*

Case	Date	Hour	Dose	Volume of Urine in 24 Hours	Protein	Plasma Chlorides	Corpuscular Volume	Hemoglobin	Remarks
			cc	cc	per cent	mM	per cent	per cent	
4	5-8-24	10 30		907	7 59	87 6	43 0	95 5	Novasurol 2 cc. at 9 0 a m
	5-9-24	10 30	2	1476	7 46	86 0	40 5	94 0	
		11 30			7 24	85 3	39 0	90 0	
		2 0			7 20	86 6	41 0	92 5	
		4 0			8 39		60 0	100 0	

TABLE 3

*The effects of novasurol administration on plasma volume and chloridion content*

Date	Time	Plasma Protein	Plasma Volume Estimated as 1/30 of the Body Weight	Plasma Chloridion	Chloridion Contained in Plasma	Remarks
		per cent	liters	mM per liter	mM	
May 13	9 30 a m	6 87	0 380	103 6	39 4	Normal dog weighing 11 4 kg given 1 cc. novasurol intramuscularly at 9 45
	10 15 a m	6 87	0 380	105 0	39 9	Shift of chloridion to plasma from tissues
	11 15 a m	6 48	0 403	106 0	42 7	Shift of chloridion to plasma from tissues
	12 15 p m	7 30	0 358	102 4	36 7	Loss of chloridion in urine
	2 15 p m	8 22	0 318	98 7	31 4	Loss of chloridion in urine
	4 15 p m	8 55	0 305	99 8	30 4	Loss of chloridion in urine
Aprl 28	10 30 a m	7 07	2 80	99 0	277 0	Case 4, given 2 cc novasurol intramuscularly at 9 a.m Apr 29
Aprl 29	9 30 a.m	6 88	2 88	98 6	284 0	Shift of chloridion to plasma from tissues
	10 30 a m	6 94	2 85	96 8	276 0	Shift of chloridion to plasma from tissues
	11 30 a m	6 92	2 86	91 9	263 0	Loss of chloridion in urine
	12 30 p m	7 05	2 81	91 9	258 0	Loss of chloridion in urine
	4 30 p m	7 63	2 59	93 4	242 0	Loss of chloridion in urine

There has been complaint occasionally of slight pain at the site of injection, which rapidly disappeared. No indurated nodules have persisted. On one occasion a small quantity of the drug escaped subcutaneously and produced extreme irritation with the subsequent development of a small necrotic area in this region. Healing, however, took place promptly. We have seen no evidence of salivation but it seems important that strict attention should be paid to the state of the mouth before and during the administration of the drug.

#### SUMMARY

Novasurol has been administered in doses of 1 to 2 cc. to eight patients suffering from heart failure. In the majority of cases decided improvement in the clinical condition took place and with one exception no untoward effect occurred. There is no evidence that any damage was done to the kidney. Diuresis commenced within the first 3 hours, reached its height in 6 to 9 hours and terminated usually in 24 hours. The output of chloride followed the same curve as the water output, but was increased to an even greater extent. The chloride in the plasma and in the edema fluid showed a fall subsequent to the injection. The fall was slightly less in the case of the edema fluid. No marked alteration was found in the blood urea or in the amount of urea or ammonia excreted. The protein content of the plasma, the corpuscular volume and the hemoglobin percentage all showed a primary fall which was maximum in  $2\frac{1}{2}$  hours and was followed by an increase which reached a greater height than that obtained before the injection.

#### DISCUSSION

In the investigation of a new therapeutic agent it is desirable to learn whether the new remedy has properties not possessed by the drugs already in common use. If it has not it is an unnecessary addition to an already overburdened list. The standard which has naturally to be applied is the evidence of clinical improvement. Too frequently we are dependent on the subjective symptoms which the patient experiences and then relates to us. In edema, however, one has the advantage of being able to study the rate of disappearance of the accumulated fluid and also the fall in weight of the patient which



accompanies its diminution. The removal of a certain amount of excess fluid from the body is of itself in most cases advantageous but even if the removal takes place it is desirable to see that there are no after effects of the drug which nullify or counterbalance the good effect which stimulating diuresis brings about. For these reasons we selected for treatment cases in the most advanced stage of heart failure in which there had been no benefit from drugs already available such as digitalis, theocin and diuretin. The results obtained in some cases were spectacular, while usually good results have been obtained. In no case has the patient's clinical condition changed for the worse.

It is necessary since novasurol contains mercury to be certain that it produces no deleterious effect on the kidneys. We have studied this point and have been unable to obtain any evidence of renal injury. From the point of view of its effectiveness, however, it appears to be better not to administer the drug more frequently than every four days. Better results are in fact obtained in this way for the amount of urine excreted tends to diminish when the drug is repeated at too short an interval. The first injection should be small in order to ascertain whether an idiosyncrasy to the drug exists.

This drug, besides having a definite action on the water output of the body, seems to have an even greater effect on its salt metabolism. We have obtained no evidence that there is any alteration in protein metabolism.

That phase of its action which has created most interest in the discussion of its pharmacology is whether the drug has its effect on the kidneys or on extra-renal tissues. The advocates of extra-renal action say that there is evidence of dilution of the blood, and conclude that there must be a primary removal of water and salt from the tissues to the blood from which it is only removed by the kidneys because of its excess in the blood. Those who oppose this view state that they can find no evidence of dilution of the blood. Our results show quite consistently that there is a primary dilution of the blood which attains its maximum within the first three hours and then that there is a swing in the opposite direction, so that for the greater part of the period during which diuresis takes place there is really a concentration of the blood. In so far as there is primary dilution of the blood, we agree with Saxl and Heilig (1922, 1923), Nonnenbruch (1921) and

Eppinger (1921) They are of opinion, however, that this is the main factor in the action of novasurol and from this circumstance are led to infer that the action must be on extra-renal tissues and that the kidneys in fact play only a passive rôle With this view we cannot agree in so far as the main action is concerned but we think their view applicable to the first stage The primary dilution of the blood might be due to one of two causes, either to the passage of water from the tissues to the blood, or to a lessened output of water by the kidneys As a matter of fact, a slightly lessened output has been observed in rare instances during the first three hours after injection, but the extent of this is never great enough to account, in itself, for the primary dilution of the blood We are, therefore, driven to the conclusion that in the early stages fluid must pass into the blood from the tissues The duration of the dilution of the blood is comparatively short and for the greater part of the period of diuresis there is concentration of the blood This is clearly shown in our determinations and has been almost invariably found by the other observers who have followed the changes in the blood for a sufficiently long period The fact that diuresis continues while the blood is concentrated seems to us incompatible with the view that the kidneys are merely removing an excess of fluid obtained from the tissues They are really playing an active part in the process

The nature of the changes which occur in the chloride of the body fluids during novasurol diuresis is of considerable interest. Here again the first response to the drug appears to be a shift of chloridion from the tissue fluids to the blood stream Then, as diuresis becomes established and a rapid excretion of chloride occurs in the urine, the chloride of the plasma quickly decreases in amount (table 3)

Determinations of the changes in the chloride concentration of the edema fluid have not been numerous enough for definite conclusions to be drawn on the mechanism which is involved in initiating the shift of salt from tissues to the blood beyond the fact that the chloride content is definitely lowered after diuresis A study of the ionic strengths of the plasma and edema fluids before and during the early shift of the water and chloridion from the tissues to the blood stream might throw some light on this interesting phenomenon

An attempt to analyse the changes occurring in salt and water metab-

olism during novasurol diuresis has been made. If one assumes that the original volume of the plasma is any definite amount, it is possible to calculate values for the changes in the plasma volume and its chloride content. These values must not be regarded as necessarily true but may be used to express relative changes from the original condition of the plasma before the administration of the diuretic. It has been assumed for the purposes of illustration (table 3) that the plasma volume was originally represented by  $1/30$  of the normal bodyweight and that the percentage of plasma protein varies inversely with the plasma volume. One can plainly observe (fig 3) an early increase in the volume of the plasma and the chloride contained in it. Then, as chloride and water were excreted in the urine, these quantities decreased rapidly and reached values much below their original level. Nevertheless, diuresis and increased chloride output were maintained in spite of an actual decrease in plasma volume and a diminution in its chloride concentration.

It seems necessary to conclude, therefore, that the drug acts not only on the kidneys but also on the tissues which contain the edema fluid. The action on the tissues is manifest for a comparatively short period only after the drug has been administered, and is shown by definite changes in the composition of the blood. Soon, however, the action on the kidneys becomes apparent, as shown not only by diuresis, but also by changes in the blood which are opposite to those occurring during the first period. During the greater part of the drug's action, indeed, the effect on the kidneys completely dominates the picture, and quite obscures the evidence of extra-renal action.

### CONCLUSIONS

1 Novasurol in doses of 1 to 2 cc injected intramuscularly or intravenously brings about marked diuresis in cases of cardiac edema which have failed to respond to other therapeutic agents.

2 Toxic symptoms are rare and transient. There was no evidence in our cases of renal irritation.

3 Diuresis commences within the first 3 hours, attains its height in 6 to 9 hours, and usually ends in 24 hours. There is a marked increase in the excretion of chloride. This action is even more marked than

that on water excretion In their time relations these two effects follow each other closely There is no evidence of any action on protein metabolism

4 The drug acts both on the kidneys and on the extra-renal tissues For a short period after administration the action on the tissues predominates, but for the greater part of the diuresis the action on the kidneys is more important.

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# A STUDY OF THE DIURETIC ACTION OF ACID PRODUCING SALTS

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## I INTRODUCTION

Since the work of Blum (1) several years ago, a number of observers (2, 3, 4) have accredited calcium chloride as an effective agent for the removal of edema fluid. In explanation of the diuretic action of this salt Blum suggested an antagonism in the body fluids between sodium and calcium with the consequence that when calcium intake is greatly raised by ingestion of a calcium containing salt, an increased excretion of sodium is produced. In support of this hypothesis, he has demonstrated an increased excretion of sodium in the urine and occasionally a measurable lowering of this base in blood plasma when  $\text{CaCl}_2$  is given in the presence of edema. Sodium retention being regarded as responsible for edema, its increased removal is supposed to permit secretion by the kidney of the super-

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\* The measurements presented in this paper were to a considerable extent obtained with the assistance of Anne C. Messer and Pauline Marsh.

† The authors are also indebted to Dr. Gerald Hoefel for the determinations of plasma pH given in table 1.

fluos body water According to the conceptions of Fischer (5), a diuresis from  $\text{CaCl}_2$  would be referred to  $\text{Ca}$  which, having in some way escaped the opposing effect of the  $\text{Cl}'$  ion, operates as base against an increased acidity of the body fluids regarded as responsible for the pathological retention of water That actually quite the inverse of such an effect follows ingestion of  $\text{CaCl}_2$  has been demonstrated by Gamble, Ross and Tisdall (6) and by Gamble and Ross (7) in their studies of the therapeutic action of several of the agents used in the treatment of infantile tetany They found  $\text{CaCl}_2$  to be in large part, from the point of view of acid-base metabolism an acid substance Following its ingestion there occurs an increase in the titratable acidity of the urine and a rise in urinary ammonia The extension of these two factors together amounts usually to about one-half the equivalence of the  $\text{Cl}'$  content of the ingested salt, so that to this extent, as regards adjustments necessary in the body fluids and in the process of acid excretion,  $\text{CaCl}_2$  taken orally is in effect  $\text{HCl}$  Measurements of  $\text{Ca}$  and  $\text{Cl}'$  in stools and urine indicate that this acid effect of ingested  $\text{CaCl}_2$  is due to a much greater absorption of  $\text{Cl}'$  than of  $\text{Ca}$  In these studies ingestion of  $\text{CaCl}_2$  was observed to produce large alterations of acid-base factors in the blood plasma and in the urine There occurred a marked reduction of plasma bicarbonate corresponding closely to an extension of chloride and accompanied by a considerable increase in plasma acidity The urine was found to contain a much increased amount of fixed base Although these infants were not edematous, these changes in the plasma and urine were found to be accompanied by a large increase in the volume of urine These findings obviously suggest the possibility of a relationship between the acid effect produced by  $\text{CaCl}_2$  and its diuretic action That this relationship exists and that the loss of body water which this salt causes is not due to some specific action by calcium was clearly indicated by the finding of Gamble and Ross (7) that  $\text{NH}_4\text{Cl}$  given to an infant with tetany caused exactly the same alterations in the blood plasma and the same increase of fixed base excretion in the urine as does  $\text{CaCl}_2$  and also produced a definite diuresis That  $\text{NH}_4\text{Cl}$  taken orally produces an acidosis had been shown by J B S Haldane (8) The acid effect of this salt is apparently due to transport of the ingested  $\text{NH}_4$  as urea, thus uncovering  $\text{Cl}'$  which claims base in the body fluids at the expense of  $\text{BHCO}_3$  In a paper published at the same time as those just cited (6, 7), Haldane, Hill and Luck (9) reported the production of marked acidosis in normal subjects by ingestion of  $\text{CaCl}_2$  They suggested that the diuretic action of this salt is probably due to its acid effect rather than to an antagonism between  $\text{Ca}$  and  $\text{Na}$  as maintained by Blum and made the surmise that  $\text{NH}_4\text{Cl}$  would be found as effective an agent for removal of edema fluid as  $\text{CaCl}_2$  In a paper recently published, Keith, Barrier and Whelan (10) report an excellent diuresis in the presence of edema in nephritis produced by giving ammonium chloride

In this paper it is desired to present data which further demonstrate that a loss of body water is in some way a consequence of ingestion of a

salt from which inorganic acid radicals enter the body fluids accompanied to a relatively slight extent by fixed base. In addition to  $\text{CaCl}_2$  and  $\text{NH}_4\text{Cl}$ ,  $\text{MgSO}_4$  and  $(\text{NH}_4)_2\text{SO}_4$  are salts which may be expected to behave in this manner and may in this sense be described as "acid producing". We have undertaken to observe and compare effects produced by ingestion of these four salts. The chief purpose of this study was to note in detail and explain if possible, alterations of acid-base values in blood plasma and in urine accompanying diuresis produced by these agents. The plan of study consisted in obtaining the desired measurements before, and again during, and following a period of salt administration, the subject receiving throughout the observation period a diet<sup>1</sup> accurately constant as regards its acid-base composition. It was further undertaken to compare findings obtained in the presence and in the absence of edema. The data to be presented are from but three children. One of them, A T a girl of 10 years, presented chronic nephritis and edema. The other two were boys, B K 8 years and J G 7 years, without edema or other evidence of renal disability. It was preferred to study the action of these several salts in the presence of edema in the same individual in order that findings might be dependably compared, which would not be the case were they obtained from several children requiring differing amounts of food and presenting various degrees of renal disease. A T proved a most suitable subject. Her chronic nephritis was of a year's duration and edema therefrom recurred gradually but regularly following removal by a diuretic agent. She remained cheerfully and healthily on the constant diet for several months and provided five periods of study. Single periods of study were obtained from the two children without edema.

<sup>1</sup> The diet was composed of cereal (Cream of Wheat) bread (salt poor), eggs, butter (salt free), milk, sugar and orange juice. The amounts given were measured by weight or volume. Of each article of food the same amount was given day by day. The cereal was cooked in a measured volume of  $\text{NaCl}$  solution which provided a usual degree of saltiness. The chloride intake was thus only moderately less than is contained in a usual diet.

The several children were given a caloric intake appropriate to their individual requirements.

The water intake was measured and was maintained at a usual level. This was for B K and J G, 1000 cc daily and for A T 1220 cc.



## II ACID PRODUCING SALTS

As mentioned above  $\text{CaCl}_2$  taken orally has been shown (6) to be physiologically an acid substance by reason of a much greater absorption of  $\text{Cl}'$  than of  $\text{Ca}$  from the gastro-intestinal tract. The similarity of certain physical properties of  $\text{Ca}$  and  $\text{Mg}$  suggests that from ingested magnesium salts the acid radical may be expected to enter the body fluids more extensively than  $\text{Mg}$ .

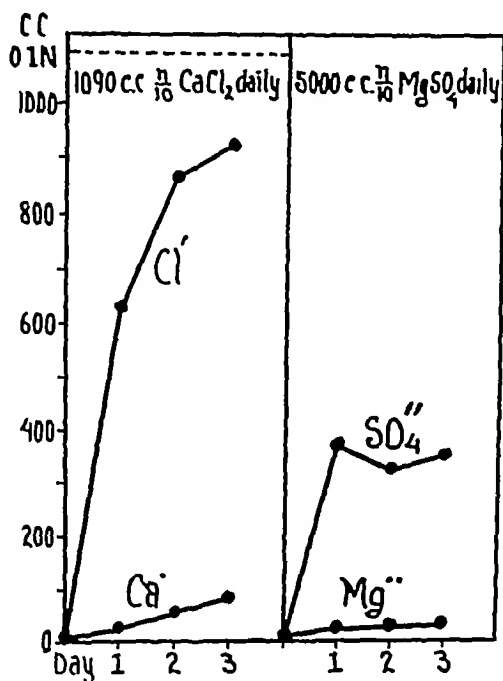


FIG 1 MEASUREMENTS OF ACID AND BASIC RADICALS IN URINE FROM INGESTED SALTS,  $\text{CaCl}_2$  AND  $\text{MgSO}_4$

Values given represent increase per 24 hours over fore period measurements

This explanation of an acid effect following ingestion of  $\text{CaCl}_2$  and of  $\text{MgSO}_4$  is illustrated by a few measurements, given in figure 1, showing the large excess of acid over basic radicals from these salts found in the urine. We may note incidentally that the excretion of  $\text{Cl}'$  rapidly approaches the increased intake indicated by the broken line in the diagram whereas the amount of  $\text{SO}_4''$  found in the urine is only a small part of the quantity ingested.  $\text{CaCl}_2$  is thus indicated as producing a larger acid effect than  $\text{MgSO}_4$ , apparently because of a less extensive absorption of  $\text{SO}_4''$  than of  $\text{Cl}'$ .

In order to clearly indicate the acid character of ingested  $\text{NH}_4\text{Cl}$ , the behaviour of the chief factors in the management of the increased excretion of  $\text{Cl}'$  following administration of  $\text{CaCl}_2$  and of  $\text{NH}_4\text{Cl}$  is compared by means of a few measurements given in figure 2. As

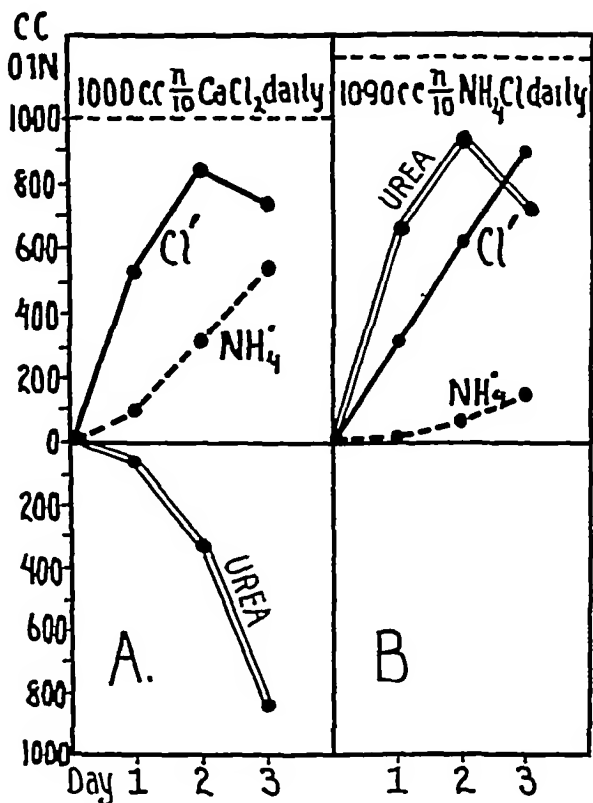


FIG 2 ILLUSTRATING AMMONIA-UREA RELATIONSHIP IN URINE FOLLOWING INGESTION OF  $\text{CaCl}_2$  AND OF  $\text{NH}_4\text{Cl}$

Urea given as cc 0.1  $\text{N}$   $\text{NH}_4$ , i.e., as twice its tenth molecular value. The values given represent increase per 24 hours over fore period measurements.

may be seen in diagram A,  $\text{CaCl}_2$  produces a rise in  $\text{Cl}'$  accompanied by a rise in  $\text{NH}_4$  at the expense of urea as indicated by the roughly proportional decline in this value. The  $\text{NH}_4$  increase is however considerably less than the extension of  $\text{Cl}'$ . In diagram B, giving the

measurements following ingestion of  $\text{NH}_4\text{Cl}$ , the rise in  $\text{NH}_4$  is even further short of the increase in  $\text{Cl}'$ , and the rise in urea indicates most of the ingested  $\text{NH}_4$  entering the urine as urea. The discrepancy between the increase in  $\text{Cl}'$  and rise in  $\text{NH}_4$  especially in the presence of edema is discussed below. These few data are presented here simply to make clear the point that ammonium salts to such extent as they are absorbed are to their full equivalence acids and in the management of their excretion the ingested  $\text{NH}_4$  is of no significance.

### III INCREASE IN PLASMA ACIDITY ACCOMPANYING THE DIURETIC ACTION OF ACID PRODUCING SALTS

Data illustrating the coincidence of plasma bicarbonate and pH lowering with a diuretic action produced by these salts, indicated by decrease in body weight<sup>2</sup> and increase in urine volume, are given graphically in figures 3 and 4. Figure 3 contains the measurements from B. K. (no edema). Calcium chloride gm 5.5 per day was given over a period of 6 days. The data from A. T. (edema) are represented by the diagrams in figure 4. She was given  $\text{CaCl}_2$ ,  $\text{NH}_4\text{Cl}$ , and  $(\text{NH}_4)_2\text{SO}_4$  in turn, in each instance following a reaccumulation of edema and over a period of four days.  $\text{CaCl}_2$  gm 6.0 was given in two 3.0 gm doses for three days. After her morning dose on the 4th day she complained of gastric discomfort and for this reason the afternoon dose was omitted. The daily dose of  $\text{NH}_4\text{Cl}$  was 5.8 gm and of  $(\text{NH}_4)_2\text{SO}_4$  7.2 gm and these amounts were given for three consecutive days. On the 4th day only the morning dose was given in order to conform with the quantity of salt given during the  $\text{CaCl}_2$  period of study<sup>3</sup>. Incidentally it may be noted that following the morning dose on the 4th day she complained in both instances of nausea, although during the preceding three days the salt had been taken without the least gastric discomfort. In terms of tenth normal solutions the addition to the intake of acid radicals provided by these amounts of

<sup>2</sup> In the case of B. K. (No Edema), body weight measurements were, regrettably, not obtained.

<sup>3</sup> Solutions of salts were prepared providing the daily intake in the following amounts of water:  $\text{CaCl}_2$ , 100 cc,  $\text{NH}_4\text{Cl}$  and  $\text{MgSO}_4$ , 200 cc. The larger amount in the case of the ammonia salts was used because of Haldane's statement that  $\text{NH}_4\text{Cl}$ , unless given in fairly dilute solution, tends to cause nausea.

the several salts was in each instance 1090 cc 0.1N per day for three days and 545 cc 0.1N during the fourth day. In the case of B. K. the acid intake was slightly less, the 5.5 gm  $\text{CaCl}_2$  providing 1000 cc 0.1N. The total excretion of inorganic acids in the urine from these children preceding ingestion of the acid producing salts was found to be about 1000 cc 0.1N daily (see tables 2, 3 and 4, Section V), i.e. this quantity of inorganic acid radicals was derived daily from the constant diet. This value is mentioned here in order to indicate the very large addition to

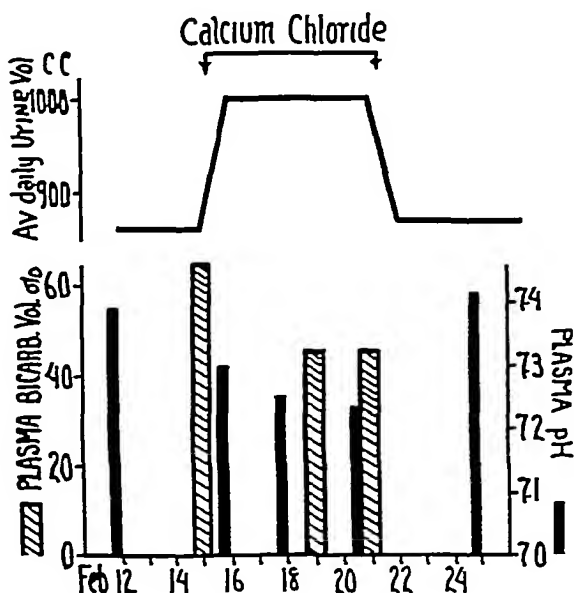


FIG 3 MEASUREMENTS FROM B. K. (NO EDEMA) ILLUSTRATING REDUCTION OF PLASMA BICARBONATE AND pH ACCOMPANYING DIURESIS PRODUCED BY INGESTION OF  $\text{CaCl}_2$

the excretion of inorganic acids caused by administering these salts in amounts corresponding to the dosage of  $\text{CaCl}_2$  usually recommended as effective in obtaining diuresis. The fact that such amounts of the salts double the quantity of acid radicals claiming excretion in the urine under usual circumstances of acid-base metabolism and provide no appreciable increase of fixed base prepares us to find without surprise a considerable alteration of acid-base adjustments within the body.

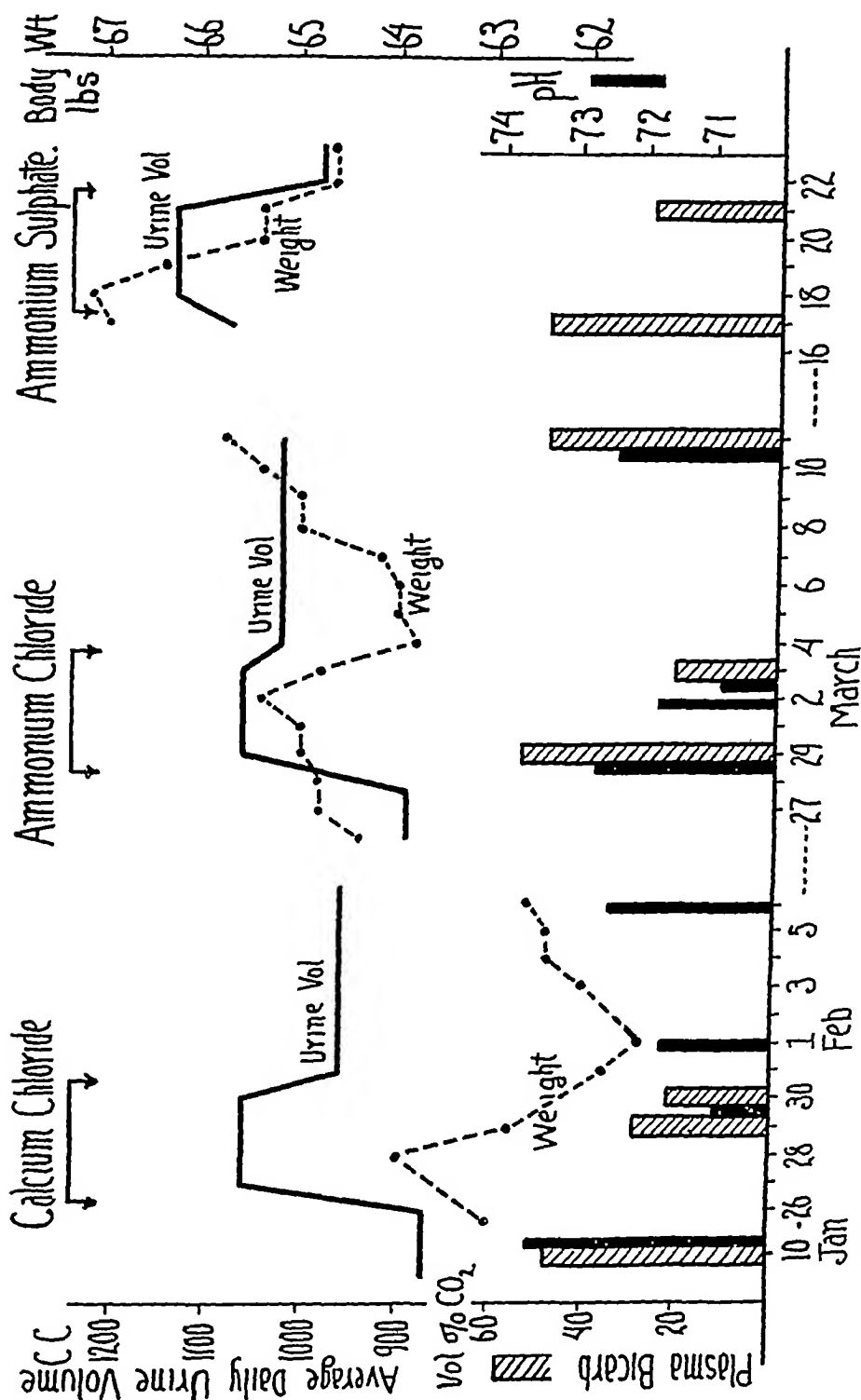


FIG 4 MEASUREMENTS FROM A T (EDEMA) ILLUSTRATING REDUCTION OF PLASMA BICARBONATE AND PH ACCOMPANYING DIURESIS PRODUCED BY INGESTION OF  $\text{CaCl}_2$ ,  $\text{NH}_4\text{Cl}$ , AND  $(\text{NH}_4)_2\text{SO}_4$

As may be seen in the diagrams, each of these salts exhibited definitely a diuretic action. The bicarbonate and pH measurements are more precisely given in the next section. They are used here simply to illustrate clearly the concurrence of diuresis with the marked acidosis which these salts produced. This relationship is so satisfactorily shown by the diagrams that further comment is unnecessary. It may here be mentioned that, so far as we are aware, an acidosis and diuretic action following ingestion of  $(\text{NH}_4)_2\text{SO}_4$  has heretofore not been described.

#### IV THE FACTORS IN THE CAUSATION OF AN INCREASED PLASMA ACIDITY BY ACID PRODUCING SALTS

Gamble, Ross and Tisdall (6) found as mentioned above that following ingestion of  $\text{CaCl}_2$  the increase of  $(\text{BCl})$  in the plasma is closely of the same extent as the decrease of  $(\text{BHCO}_3)$ . In agreement with this finding they also demonstrated that total fixed base in the plasma is not appreciably changed by administration of this salt. The reduction of bicarbonate is thus practically entirely referable to an extension of  $(\text{Cl}')$  which dispossesses  $(\text{HCO}_3')$  of an equivalence of base. Haldane, Hill and Luck, in their paper appearing at the same time, also noted the close equivalence of the chloride increase and bicarbonate reduction in calcium chloride acidosis. Gamble and Ross (7) noted that  $\text{NH}_4\text{Cl}$  given an infant with tetany produced closely reciprocal changes in  $(\text{BCl})$  and  $(\text{BHCO}_3)$  in the plasma and that indicated by stationary values for  $(\text{Na})$ , there was no appreciable loss of plasma base.

Data explaining the bicarbonate reduction seen in figures 3 and 4 are given in table 1, and are also presented graphically by means of the diagrams in figure 5. The measurements (except those of pH) are given as cc 0.1% per 100 cc of plasma in order that they may be compared in terms of acid-base equivalence.<sup>4</sup> It is believed that the

<sup>4</sup> The actual amount of univalent base bound in the plasma is  $1.8 \times (\text{HPO}_4)$ , not twice the equivalence of the concentration of this radical as is suggested by the double valency symbol. This value is easily derived from the fact that at pH 7.4 20% of  $(\text{HPO}_4')$  is bound as  $\text{BH}_2\text{PO}_4$  and 80 per cent as  $\text{B}_2\text{HPO}_4$ . The base equivalence of  $(\text{HPO}_4')$  is thus  $0.2 + (2 \times 0.8) = 1.8$ . This factor was used in obtaining the values given in the table and diagrams. It will be understood that

diagrams exhibit more clearly and in better perspective than tabulated data the parts of the ionic structure in the plasma and the manner of their interdependence. The left hand column in each diagram represents the fixed base measurement. Laid off against this successively beginning at the top are the measurements of such acid values as were obtained. The unmeasured acid values are contained in the remainder of the acid column designated R. These are three,  $\text{SO}_4''$ , organic acids, and the base binding equivalence of the plasma proteins.<sup>5</sup> Measure-

TABLE 1

*Showing alterations of certain acid-base values in blood plasma caused by ingestion of acid producing salts*

	B K (no edema)		A T (chronic nephritis and edema)					
	Effect of $\text{CaCl}_2$		Effect of $\text{CaCl}_2$		Effect of $\text{NH}_4\text{Cl}$		Effect of $(\text{NH}_4)_2\text{SO}_4$	
	Before	After*	Before	After	Before	After	Before	After
	$\frac{\text{cc}}{\text{O I N}^\dagger}$	$\frac{\text{cc}}{\text{O I N}}$	$\frac{\text{cc}}{\text{O I N}}$	$\frac{\text{cc}}{\text{O I N}}$	$\frac{\text{cc}}{\text{O I N}}$	$\frac{\text{cc}}{\text{O I N}}$	$\frac{\text{cc}}{\text{O I N}}$	$\frac{\text{cc}}{\text{O I N}}$
(Fixed base)	154 0	150 0	151 0	153 0	156 0	156 0	157 0	155 0
(Ca )	4 5		4 2	4 5	4 0	4 1		
(Cl')	103 0	112 0	114 0	124 0	111 0	124 0	111 0	120 0
( $\text{HCO}_3'$ )	28 8	20 0	20 7	9 4	23 7	9 4	21 6	11 9
(Cl') + ( $\text{HCO}_3'$ )	131 8	132 0	134 7	133 4	134 7	133 4	132 6	131 9
( $\text{HPO}_4'$ )	2 7	2 5		3 5	3 1	3 4	3 1	3 5
( $\text{SO}_4''$ )							1 3	1 2
pH	7 37	7 32	7 18	7 08	7 27	7 07	7 27	—

\* Measurements in the columns marked "After" were obtained at the end of the 3rd day of salt administration

† Per 100 cc of plasma

the measurements of ( $\text{HCO}_3'$ ) as determined by the Van Slyke method for plasma bicarbonate do not contain the approximately 1/20 of the total ( $\text{HCO}_3'$ ) in the plasma which is present as  $\text{H HCO}_3$ , so that as indicated these values for ( $\text{HCO}_3'$ ) cover a full equivalence of base. The base equivalence of (Cl') is of course 1 0 and that of ( $\text{SO}_4''$ ) is 2 0

<sup>5</sup> As has been noted (4, page 367), among the three inorganic acid radicals only  $\text{HPO}_4''$  binds slightly less than its full valency equivalence of base in the plasma. (Cl') and ( $\text{SO}_4''$ ) are fully covered by base both in the plasma and in urine. ( $\text{HPO}_4'$ ) carries still less of base into acid urine than it binds in the plasma. By expressing the acid excretion in terms of base bound while being conveyed in the body fluids, an increase may be correctly compared with an accompanying increase in excretion of  $\text{NH}_4$  as a means of estimating fixed base withdrawal.

ments of  $\text{SO}_4''$  are given in the last pair of diagrams. Adjustment of the acid side of this structure to the fixed base level is an automatic consequence of the elasticity of  $(\text{HCO}_3')$  and for this reason this value is appropriately placed at the top of the acid column. The acid side being adjustable the importance of an accurate maintenance of fixed base from the point of view of preserving a correct total ionic content of the plasma is apparent.

In table 1 and in figure 5 it may be seen that in spite of the huge addition to the excess of acid over fixed base claiming excretion in the urine the plasma fixed base remains closely stationary. It is not desired to intimate that the just measurable differences in this value are without significance. It is probable that small changes may considerably alter processes dependent on osmotic pressure adjustments. A discussion of these few data from this point of view is, however, not warranted here. As regards the large reduction of bicarbonate which evidently alters the  $(\text{BHCO}_3)$   $(\text{H}_2\text{CO}_3)$  ratio to an extent producing a marked increase of plasma acidity the measurements indicate that this change is not to appreciable extent caused by a depletion of plasma base. The relationship of  $(\text{BHCO}_3)$  reduction to the large rise in the level conveyance  $(\text{Cl}')$  is shown in the table by the nearly stationary values for the sum of  $(\text{HCO}_3')$  and  $(\text{Cl}')$ , and may be seen at a glance in the diagrams. The acidosis produced by ingestion of  $\text{CaCl}_2$  or of  $\text{NH}_4\text{Cl}$  is thus, as might be expected, understandable as a direct consequence of the greatly increased quantity of  $\text{Cl}'$  demanding transport. It could scarcely have been anticipated however that a reduction of  $(\text{BHCO}_3)$  following ingestion of  $(\text{NH}_4)_2\text{SO}_4$  would also be found to be caused by an equivalent extension of  $(\text{Cl}')$ . The measurements of  $(\text{SO}_4'')$  were made with the expectation that increase in this value might explain the lowering of  $(\text{BHCO}_3)$ . As may be seen however in table 1 and figure 5 the relatively very small concentration at which  $(\text{SO}_4'')$  is carried in the plasma was not measurably altered by administration of  $(\text{NH}_4)_2\text{SO}_4$  in spite of the fact that, during this period, about four times as much  $\text{SO}_4''$  was carried into the urine per day as during the fore period (see table 4, Section 5). That the immediate cause of reduction of  $(\text{BHCO}_3)$  after giving  $(\text{NH}_4)_2\text{SO}_4$  is an extension of  $(\text{Cl}')$  just as occurs following ingestion of  $\text{CaCl}_2$  or  $\text{NH}_4\text{Cl}$  is clearly apparent in the diagrams in figure 5. This large increase in  $(\text{Cl}')$ , related in



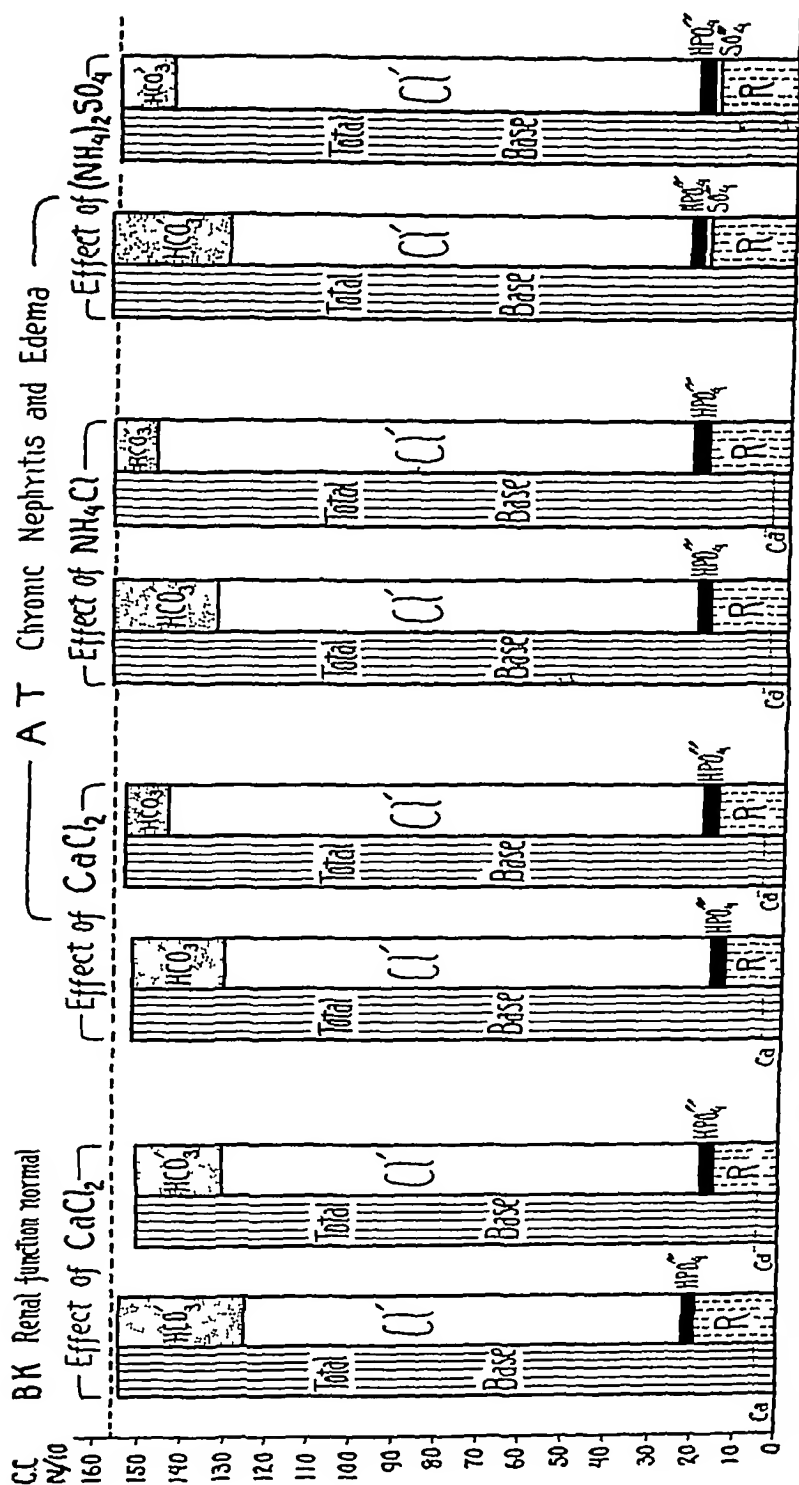


FIG. 5 ILLUSTRATING CHANGES PRODUCED IN ACID-BASE COMPOSITION OF BLOOD PLASMA BY INGESTION OF  $\text{CaCl}_2$ ,  $\text{NH}_4\text{Cl}$ , AND  $(\text{NH}_4)_2\text{SO}_4$

Diagrams constructed from measurements given in table 1

some way to an increased metabolism of  $\text{SO}_4^{''}$  must be of considerable significance as regards the rôle of  $(\text{Cl}')$  in the mechanism managing acid-base adjustments in the body fluids. This significance however, is not easily discernible and it is desired here simply to report this finding without surmise as to its import.

Measurements of  $(\text{HPO}_4^{''})$  were obtained to cover the possibility that phosphate retention might be a factor in  $(\text{BHCO}_3)$  reduction in the case of the nephritic child A. T. No appreciable increase of this plasma acid was found. As regards the extent to which phosphate retention may be a factor in producing acidosis, it should be appreciated that a relatively very large increase in  $(\text{HPO}_4^{''})$  would be necessary in order to cause any considerable reduction of  $(\text{BHCO}_3)$ . If the usual magnitudes of these values as shown in figure 5 be compared, it will be apparent that  $(\text{HPO}_4^{''})$  must increase several fold to cause a moderate reduction of  $(\text{BHCO}_3)$ . In contrast, a 20 per cent increase in  $(\text{Cl}')$  will lower  $(\text{BHCO}_3)$  by more than one-half of its usual value. Marriott and Howland (11) and later, Denis and Minot (12) have published measurements of phosphate retention in nephritis a few of which are of a magnitude sufficient to reduce a normal  $(\text{BHCO}_3)$  by one-third or even by one-half. Such values were, however, nearly always obtained in the terminal and uremic phase of the disease.

The calcium measurements given in table 1 demonstrate that this base factor in the plasma is not appreciably altered by ingestion of large amounts of a calcium containing salt. It remains somewhat below its usual value which may be taken as 5.0 cc 0.1N per 100 cc of plasma. This lowering of  $(\text{Ca})$  is a usual finding in nephritis with edema. The slight extent to which the fixed base of the plasma is composed of  $(\text{Ca})$  is indicated in the diagrams (figure 5). That reduction of  $(\text{BHCO}_3)$  produced by these salts in the manner just described is accompanied by a marked increase in plasma acidity is shown by the direct measurements of plasma pH given in table 1.

An important point appearing in these data is the fact that the changes in the plasma produced by these salts, viz., increase in  $(\text{Cl}')$  reducing  $(\text{BHCO}_3)$  and thus increasing acidity, were found to be already established to a considerable degree in the case of A. T., the child with nephritis and edema, *before* the salts were given. The degree of chloride acidosis presented by A. T. (edema) before ingestion

of the salts may be appreciated by a glance at the diagrams in figure 5. For instance, before  $\text{CaCl}_2$  was given,  $(\text{Cl}')$  was found increased and  $(\text{HCO}_3')$  reduced to approximately the extent found in the plasma from B K (no edema) after ingestion of this salt, as may be seen by comparing these adjacent diagrams. Since an increased acidity of the plasma brought about by a rise in  $(\text{Cl}')$  is found to be accompanied by an increased rate of removal of water from the body, it is perhaps not unreasonable to regard the unusually high  $(\text{Cl}')$  observed in the plasma of A T, not as a retention in the pathological sense, but as a beneficial adjustment in the presence of an obstacle imposed by disease. As possibly supporting this interpretation of the increased  $(\text{Cl}')$  it may be pointed out that the resultant rise in plasma acidity must be regarded as surprisingly large if our customary assumption that even very considerable reductions of  $(\text{BHCO}_3)$  are closely compensated for by respiratory adjustment is correct. As further comment in this direction, it may be suggested that the finding of an altered factor in the plasma should not constitute an unqualified indication to restore it by some direct means to its usual value. At any rate in this instance in the presence of an acidosis, not bicarbonate, but a salt increasing the degree of plasma acidity, produces an apparently desirable therapeutic result, viz, a removal of superfluous body water.

#### V THE INCREASE IN FIXED BASE EXCRETION IN THE URINE AND BEHAVIOR OF THE FACTORS OF ACID EXCRETION FOLLOWING INGESTION OF ACID PRODUCING SALTS

The increase in the excess of acid over fixed base claiming excretion in the urine caused by ingestion of acid producing salts must obviously be accompanied by an equivalent extension of the factors which spare the use of fixed base in the process of acid excretion if withdrawal of fixed base from the body is to be prevented. The conveyance of an acid excess into the urine is, as has been shown by Henderson (13) and Henderson and Palmer (14), undertaken by the operation together of two adjustments, a regulated production of ammonia at the expense of urea and an absolute saving of base obtained by secretion of urine at a pH lower than that of blood plasma. Ammonia production is usually the larger and is also much the more extensible of these two factors. Under the circumstances present in these studies, increase in

ammonia production is practically the only adjustment available during the periods of increase in acid excess for the reason that the fore period urines are of a reaction near the physiological limit of acidity so that no appreciable increase in base saving is obtainable by the slight further lowering of urine pH which is possible. A glance at the few data represented in the diagrams in figure 2 will serve to clearly indicate the fact that extension of the ammonia factor falls far short of the increase in excretion of the acid radical of the ingested salt. It may be correctly inferred from these data that the discrepancy between the increase of  $\text{Cl}'$  and that of  $\text{NH}_4$  indicates a withdrawal of fixed base from the body. We should not, however, regard  $\text{Cl}' - \text{NH}_4$  as accurately measuring the increase in fixed base excretion without first testing the possibility of minor alterations in the metabolism levels of the other acid radicals in the presence of the large increase in the intake of  $\text{Cl}'$ . In order to determine fairly completely all of the factors in the situation, the following measurements were obtained from 24-hour urine specimens collected before, during and following the periods of salt administration: volume, pH, inorganic sulphates and phosphates, chlorides, ammonia, fixed base, and as a means of estimating the accuracy of collection of the 24-hour urine specimen, creatinine. Because of the presence of albumin in the specimens from A T (edema), measurements of the titratable acidity of the urine and of the organic acid excretion were not undertaken. As has just been mentioned, the base-saving factor which is measured by the titratable acidity of the urine is not appreciably operative in covering the increase in acid excess produced by the salts. These data are contained in tables 2, 3, and 4, the measurements of base being given as cc 0.1N and those of the inorganic acid radicals as cc 0.1N of base equivalence at the reaction of blood plasma. Except for those obtained during a period of  $\text{MgSO}_4$  administration to A T and given in the upper section of table 4, the measurements in these tables were obtained during the periods of study which supplied the data given in the preceding section. Attention may perhaps be first directed to certain small changes in the excretion levels of the acids other than the one contained in the ingested salt. As may be seen in table 3-A, the large increase in  $\text{Cl}'$  in urine during ingestion of  $\text{CaCl}_2$  by A T is accompanied by definite decrease in  $\text{HPO}_4$  excretion and also a just discernible reduction of

$\text{SO}_4'$  The increase in total acid excretion due to  $\text{Cl}'$  is thus in this instance to a slight extent offset by a decrease in  $\text{HPO}_4'$  and  $\text{SO}_4'$ . These changes can be reasonably explained as due to a reduction of  $\text{HPO}_4''$  and  $\text{SO}_4''$  absorption caused by an increased formation of insoluble calcium phosphates and sulphates in the intestine. They are not, however, distinctly apparent in the urines from B K when the same salt was given (see table 2). As would be expected on the basis of the surmise just offered, administration of  $\text{NH}_4\text{Cl}$  did not lower the excretion levels of  $\text{HPO}_4''$  and  $\text{SO}_4''$  (see table 3-B). In table 4 may be

TABLE 2

*B K (no edema) Calcium chloride period of study Measurements from 24-hour urine specimens*

Day	CaCl <sub>2</sub> Ingested	Urine volume	Creat- inine	pH	$\text{SO}_4'$	$\text{HPO}_4'$	$\text{Cl}'$	$\text{NH}_4$	Fixed base	Increase over fore period		
										$\text{Cl}'$	$\text{NH}_4$	Fixed base
	cc O I N	cc	mg		cc O I N	cc O I N	cc O I N	cc O I N	cc O I N	cc O I N	cc O I N	cc O I N
1-4	0	860	355	5.9	236	357	354	228	548			
1	1000	1040	360	5.0	229	317	891	326	931	534	98	383
2	1000	1060	398	4.7	246	348	1191	549	1072	837	321	524
3	1000	930	348	5.0	231	316	1086	776	686	732	548	138
4	1000	1100	366	5.1	265	338	1257	840	745	903	612	197
5	1000	860	370	5.3	233	238	1186	986	566	832	758	18
6	1000	1010	418	5.3	313	335	1257	1123	651	903	895	103
1	0	940	325	6.2	252	299	700	928	250	346	700	-298
2	0	670	333	6.0	280	306	393	680	171	39	452	-377
3	0	910	349	6.0		319	371	461	306	17	233	-242
4	0	800	333	5.9		343	450	325	584	96	97	36

seen during the first several days of the  $\text{MgSO}_4$  and  $(\text{NH}_4)_2\text{SO}_4$  periods a fairly large increase in  $\text{Cl}'$ . An explanation of this event will be undertaken below. As indicated in figure 1, the fixed base excretion contains only to a slight extent Ca or Mg from the ingested salt. All of these changes just noted are, however, relatively small. The increase in total acid excretion may therefore be approximately determined by measuring the increase in the urine of the acid radical of the ingested salt and, by subtracting from this value the ammonia increase, the withdrawal of fixed base from the body may be fairly

closely estimated. We were however, at pains to directly measure the fixed base excretion by the method of Fiske. The chief purpose of

TABLE 3

Data from 1 *T. edentata*. Calcium chloride and ammonium chloride periods of study.  
Measurements from consecutive 24 hour urine specimens

Day	Salt given	Urine volume	Creatinine	pH	SO <sub>4</sub>	HPO <sub>4</sub>	Cl	NH <sub>4</sub>	Fixed base	Increase over fore period		
										Cl	NH <sub>4</sub>	Fixed base
A CaCl period												
1-3	0	870	525	5.0	196	331	443	339	530			
1	1090	1000	520	5.0	164	164	1071	336	974	628	-3	444
2	1090	1130	544	5.0	205	205	1307	461	1088	864	122	558
3	1090	990	520	5.0	196	258	1357	518	1394	914	179	864
4	545	1140	528	5.0	156	248	1018	488	864	575	149	334
1	0	970	521	5.0	221	315	686	586	576	243	247	46
2	0	870	578	5.1	261	342	650	600	572	207	261	42
3	0	1040	506	5.2	235	311	471	721	523	28	382	-7
4	0	910	470	5.2	220	306	529	529	461	86	190	-69
5	0	950	470	5.2	194	324	479	475	471	36	136	-59
6	0	815	525	5.1	194	313	457	407	495	14	68	-35
B NH <sub>4</sub> Cl period												
1-3	0	870	450	4.6	215	340	390	261	562			
1	1090	1000	540	4.6	217	331	700	266	862	310	5	300
2	1090	980	540	4.6	218	325	1007	342	1086	617	81	524
3	1090	1200	522	4.9	217	359	1286	435	1388	896	174	826
4	545	890	580	4.9	197	334	807	438	872	417	177	310
1	0	975	555	4.9	209	331	700	486	632	310	225	70
2	0	1050	522	4.9	202	305	634	520	608	244	259	46
3	0	960	555	4.9	201	322	586	452	570	196	191	8
4	0	895	540	4.9	187	325	521	425	526	131	164	-36
5	0	1130	522	4.9		369	463	414	468	73	153	-94
6	0	1000	540	4.8	182	331	393	361	544	3	100	-18

these data being to illustrate the manner and extent of fixed base removal from the body during administration of acid producing salts,

the three essential measurements are those of the increase in urine of the acid radical of the ingested salt, ammonia, and fixed base, over fore period values. The values found for these three factors are given in heavy faced type in the last three columns of the tables. In order to make their relationship easily apparent, they are, except for the

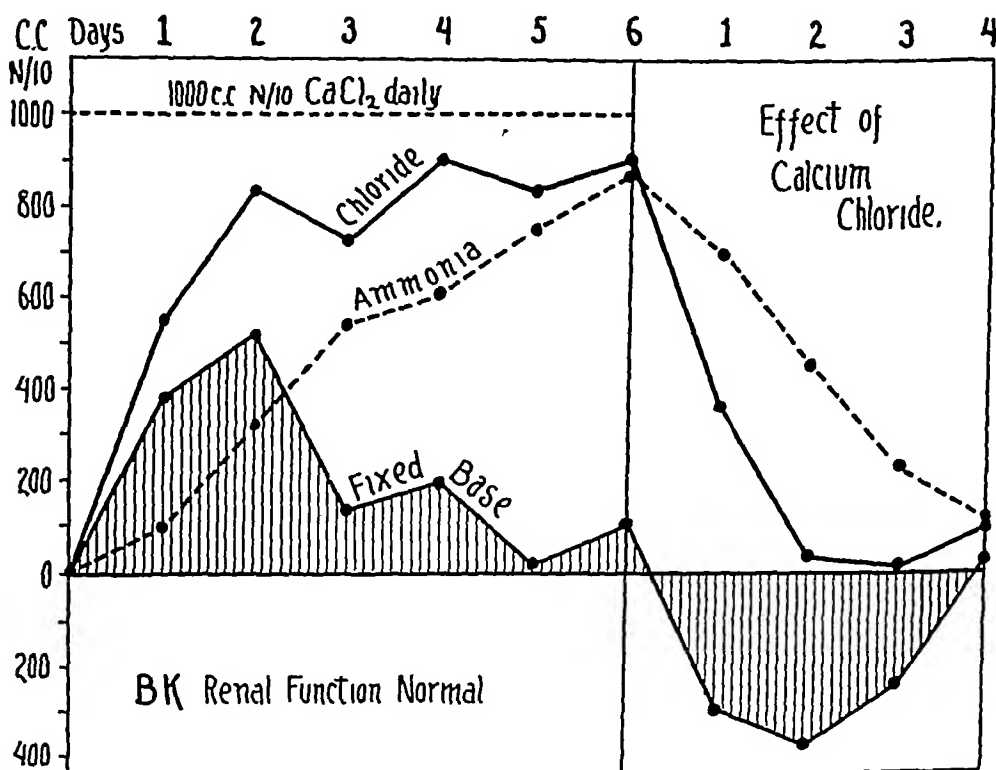


FIG 6 DATA FROM B K (NO EDEMA) REPRESENTING FACTORS DETERMINING AN INCREASED EXCRETION OF FIXED BASE IN URINE DURING A 6-DAY PERIOD OF  $\text{CaCl}_2$  ADMINISTRATION

Measurements are those of increase over fore period values given in heavy faced type in table 2

measurements obtained during the  $\text{MgSO}_4$  period, also presented graphically by means of the diagrams in figures 6 and 7. The base line in these diagrams is the fore period level, increase being plotted above and decrease below this line. Diagrams presenting the results of two additional periods of study are given in figure 8. These were obtained from A T (edema) and J G (no edema) before, during, and

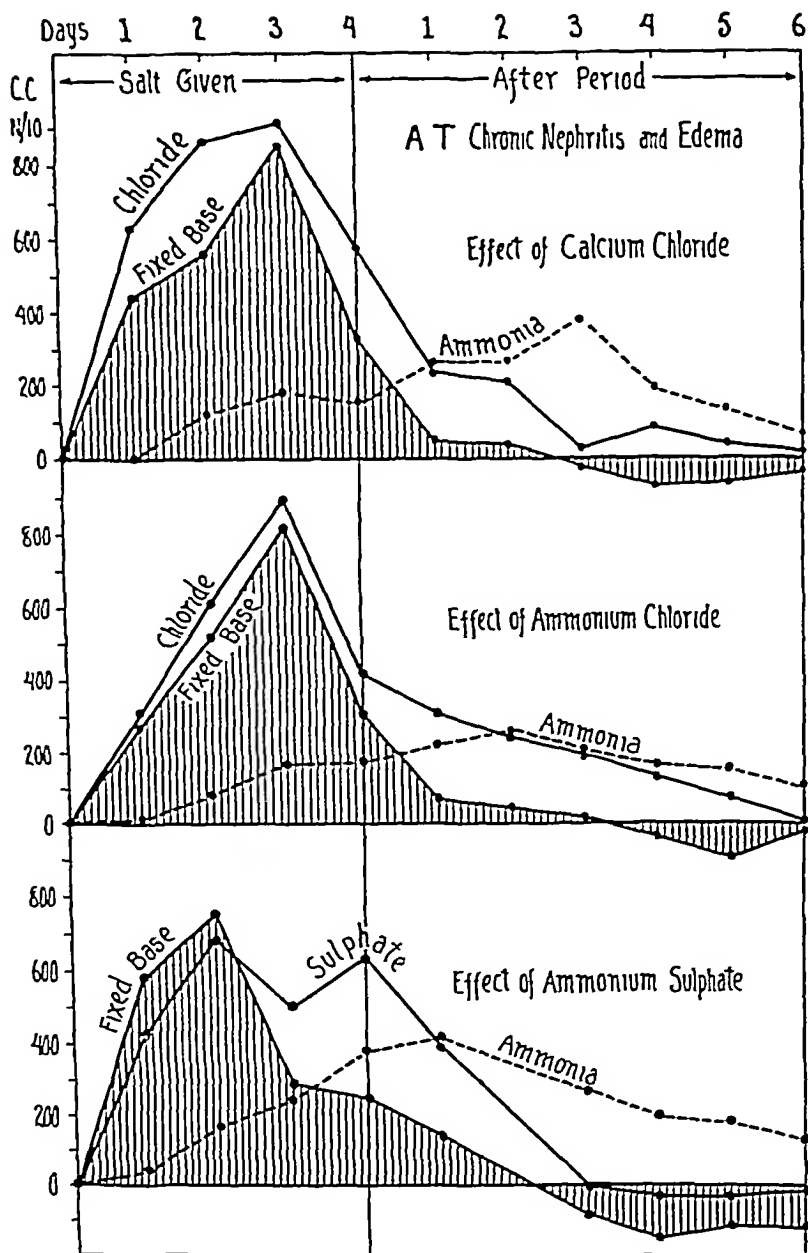


FIG 7 DATA FROM A T (EDEMA) REPRESENTING FACTORS DETERMINING AN INCREASED EXCRETION OF FIXED BASE IN URINE DURING 4-DAY PERIODS OF INGESTION OF  $\text{CaCl}_2$ ,  $\text{NH}_4\text{Cl}$ , AND OF  $(\text{NH}_4)_2\text{SO}_4$

Measurements are those of increase over fore period values given in heavy faced type in tables 3 and 4-B



following 8-day periods of administration of  $\text{NH}_4\text{Cl}$ , gm 30, per day, i e, approximately one-half the amount of salt given A T during the period already described

The diagrams will, it is believed, make unnecessary more than brief comment on the urine findings. The rough agreement of fixed base withdrawal with the extent to which  $\text{NH}_4$  fails to cover the acid increase is easily apparent. Attention may be called to the nearly identical character of the diagrams in figure 7 representing changes in the three factors produced by ingestion of equivalent amounts of  $\text{CaCl}_2$  and of  $\text{NH}_4\text{Cl}$ . The fact that there is essentially no difference in the manner and extent of base withdrawal caused by these salts would seem to quite clearly indicate that, in the case of  $\text{CaCl}_2$ , Ca plays no rôle in this action. The lower diagram in the figure illustrating the changes produced by an equivalent amount of  $(\text{NH}_4)_2\text{SO}_4$  fairly closely resembles those of the acid chloride periods. The fact that the fixed base points at first lie above those measuring the  $\text{SO}_4''$  increase is explained by an accompanying increase in  $\text{Cl}'$  during the first two days of the period (see table 4). Absorption of  $\text{SO}_4''$  when given as  $(\text{NH}_4)_2\text{SO}_4$  is thus seen to be practically as extensive as that of  $\text{Cl}'$  from  $\text{CaCl}_2$  or  $\text{NH}_4\text{Cl}$ . In contrast may be noted the much smaller amounts of  $\text{SO}_4''$  found in the urine when  $\text{MgSO}_4$  in much larger dosage was given (see table 4). This difference is in part, but probably not entirely, due to the fact that the large amount of  $\text{MgSO}_4$  given produced copious catharsis. It may be mentioned here that during none of the other periods of study did the salts in the amounts used cause catharsis. The data in table 4-A are given simply to demonstrate that  $\text{MgSO}_4$  is to slight extent an acid producing salt and, in addition to its action as an intestinal hydragogue, causes also to a measureable extent a removal of fixed base by way of the kidney. As regards a probable practical usefulness of  $(\text{NH}_4)_2\text{SO}_4$  as a diuretic agent, it may be further mentioned that in solution it is very much less disagreeable tasting than  $\text{CaCl}_2$  or  $\text{NH}_4\text{Cl}$ .

Another chief point which these data demonstrate is that the acid producing salts when given the child with nephritis and edema caused a more rapid and longer sustained removal of fixed base than occurred when such a salt was taken by B K or by J G, the children without edema or renal disease. This difference may be seen at a glance by

comparing the diagrams (figures 6 and 7) representing the measurements obtained during the  $\text{CaCl}_2$  periods of B K (no edema) and A T

TABLE 4

Data from A T (edema) Magnesium sulphate and ammonium sulphate periods of study  
Measurements from consecutive 24 hr urine specimens

Day	Salt given	Urine volume	Creat urine	pH	SO <sub>4</sub>	HPO <sub>4</sub>	Cl	NH <sub>4</sub>	Fixed base	Increase over 24 hr period.		
										Cl	NH <sub>4</sub>	Fixed base
A MgSO <sub>4</sub> period												
1-3	0	840	490	4.9	212	302	428	196	674			
1	5000	940	500	4.8	583	290	379	230	948	371	36	274
2	5000	1125	495	4.8	532	331	839	275	1194	320	79	520
3	5000	720	480	4.8	561	363	529	317	748	349	121	74
4	5000	710	475	4.9	448	328	543	375	616	236	179	-58
5	2500	715	475	4.9	364	305	321	375	562	152	179	-112
6	2500	815	505	4.9	583	325	400	404	808	371	208	134
7	2500	720	475	4.9	647	305	357	367	851	435	171	177
8	2500	760	505	4.9	773	305	357	379	889	561	183	215
1-3	0	726	500	5.0	396	342	386	436	600	183	240	-74
B (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> period												
1-3	0	1070	520	4.4	204	320	370	187	521			
1	1090	1200	564	4.4	636	331	489	232	1104	432	45	583
2	1090	1280	572	4.4	892	358	520	358	1277	688	171	756
3	1090	1025	523	4.8	705	319	306	433	814	501	246	293
4	545	1030	550	4.8	843	364	306	565	772	639	378	251
1	0	1115	545	5.0	602	338	403	591	662	398	404	141
2*	0											
3	0	900	575	5.2	199	319	457	458	438	-5	271	-83
4	0	960	515	5.2	171	290	400	382	372	-33	195	-149
5	0	1000	495	5.2	175	296	386	370	406	-29	183	-115
6	0	920	525	5.0	186	288	386	323	400	-18	136	-121

\* Collection of 24-hour specimen was incomplete.

(edema), and the immediate cause of the larger withdrawal of fixed base from A T is at once discernible in the sluggish rise of the line

measuring  $\text{NH}_4$  increase in contrast with its rapid ascent in the case of B K, the rate of acid increase in the urine being approximately the same for both subjects. The differences in the behaviour of the three factors in the presence and in the absence of nephritis and edema during

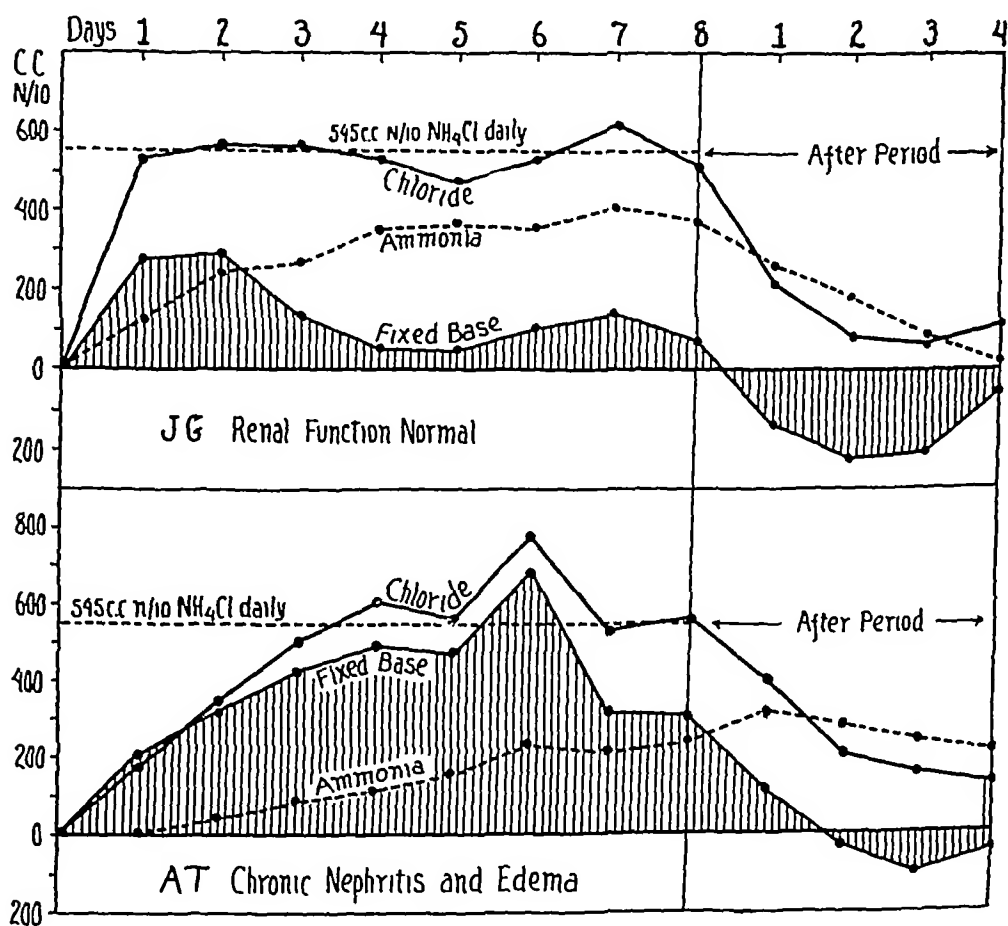


FIG 8 DATA FROM J G (NO EDEMA) AND A T (EDEMA) REPRESENTING FACTORS DETERMINING AN INCREASED EXCRETION OF FIXED BASE IN URINE DURING 8-DAY PERIODS OF  $\text{NH}_4\text{Cl}$  ADMINISTRATION

Measurements are of increase per 24 hours over fore period values

longer periods of ingestion of smaller amounts of salt, in this case  $\text{NH}_4\text{Cl}$ , by A T (edema) and by J G (no edema) are shown by the diagrams in figure 8. The slower rise in ammonia production and in consequence the much larger loss of fixed base in the case of A T (edema) are here seen again. In these diagrams may be noted a

difference in the rate of  $\text{Cl}'$  removal which does not appear in the data from the  $\text{CaCl}_2$  periods of A T and B K. Here in contrast with the prompt appearance of the added  $\text{Cl}'$  intake in the urine from J G (no edema) the increase in  $\text{Cl}'$  excretion mounts gradually in the case of A T (edema) the initial lag however being made up for by an ultimate rise above the level of added intake indicated by the broken line across the chart. To compare in total values the loss of base from these two children, the sum of the daily measurements of increase in fixed base excretion during the 8 days of  $\text{NH}_4\text{Cl}$  administration is 1129 cc 0.1N for J G (no edema) and 3332 cc 0.1N for A T (edema). During the 4-day after periods J G regained 613 cc 0.1N and A T only 160 cc 0.1N.

Having indicated the manner and extent of the increased excretion of fixed base in the urine during the periods of administration of acid producing salts it is obviously necessary to undertake to explain the closely stationary concentration of fixed base in blood plasma shown by the data given in the preceding section. The terms of the situation practically demonstrate its explanation. Assuming a maintenance of absolutely stationary concentrations of fixed base in the body fluids in the presence of a removal of fixed base into the urine, it is only possible to infer an accurately proportional reduction of the volume of the body fluids from which the fixed base loss derives. The measurements of plasma base given in Section III, while regarded as demonstrating only an approximately stationary concentration, are nevertheless so near the usual value as to indicate with certainty a close relationship between the loss of body water and the increased excretion of fixed base. Data illustrating the closely proportional withdrawal of body water accompanying the excretion of fixed base in urine during periods of fasting have been published by Gamble, Tisdall and Ross (15). It is therefore permissible to regard the increase in excretion of fixed base in urine shown above as roughly measuring the diuretic action of the acid producing salts. The increase of  $\text{Cl}'$  in the urine accompanying the diuretic action of  $\text{MgSO}_4$  or of  $(\text{NH}_4)_2\text{SO}_4$  can be satisfactorily explained in terms of the conception here being used. At least in blood plasma,  $\text{Cl}'$  is the largest acid factor, covering about three-fifths of the total plasma base (see diagrams in figure 5). A loss of body water, especially if chiefly from

the extra-cellular compartment, should therefore produce a considerable increase in the amount of  $\text{Cl}'$  entering the urine. A withdrawal of  $\text{Cl}'$  also occurs during the acid chloride periods. For instance it may be computed from the data used in constructing the diagrams in figure 8 that the increase of  $\text{Cl}'$  in the urine for the 12-day period was actually more than the total of increased intake during the 8 days that  $\text{NH}_4\text{Cl}$  was given. The values thus obtained are increased intake (both subjects) 4760 cc 0.1N, increased excretion J. G. (no edema) 4827 cc 0.1N and A. T. (edema) 4974 cc 0.1N. The surplus of  $\text{Cl}'$  excretion over intake is thus larger in the case of A. T. (edema) corresponding appropriately to a larger removal of body water. Incidentally these data indicate an unimpaired ability of the kidney in the case of the nephritic child to eliminate  $\text{Cl}'$  and support the surmise that the increase of ( $\text{Cl}'$ ) found in the plasma before administration of the salts (Section IV) should be regarded as an adjustment and not as a fault of renal function. These data also indicate the probability that the tendency to edema which ingestion of  $\text{NaCl}$  sometimes produces in nephritis is referable to  $\text{Na}$  and not to  $\text{Cl}'$  and suggest that nephritic diets might safely and also beneficially, but unfortunately not palatably, contain the acid chlorides.

#### VI COMPOSITION OF FIXED BASE EXCRETION IN URINE FOLLOWING INGESTION OF CALCIUM CHLORIDE

The fixed base concentrations of the body fluids are composed chiefly of ( $\text{Na}$ ) and ( $\text{K}$ ), the former constituting nearly all of the base in extra-cellular water and the latter at least two-thirds of the base in intra-cellular water.<sup>6</sup> Regarding an increase in fixed base excretion as consisting of the base content of a portion of body water which has been removed, analysis of its composition, especially as regards  $\text{Na}$  and  $\text{K}$ , should serve to indicate the source of the withdrawn water. The amounts of  $\text{K}$ ,  $\text{Ca}$  and  $\text{Mg}$  in the urine specimens collected during the calcium chloride periods of study from both B. K. (no edema) and A. T. (edema) were directly determined and a value for

<sup>6</sup> This statement is based on a comparison of the concentrations of the four bases in the water of blood plasma and of muscle tissue contained in a paper by Gamble, Ross and Tisdall (15) discussing fixed base metabolism.

$\text{Na}^+$  was obtained by subtracting the three other bases from the total fixed base measurement. These data are given in figure 9. Interpreting the measurements of  $\text{Na}^+$  and  $\text{K}^+$  according to the premise just suggested, the diagrams indicate a removal of water from both the

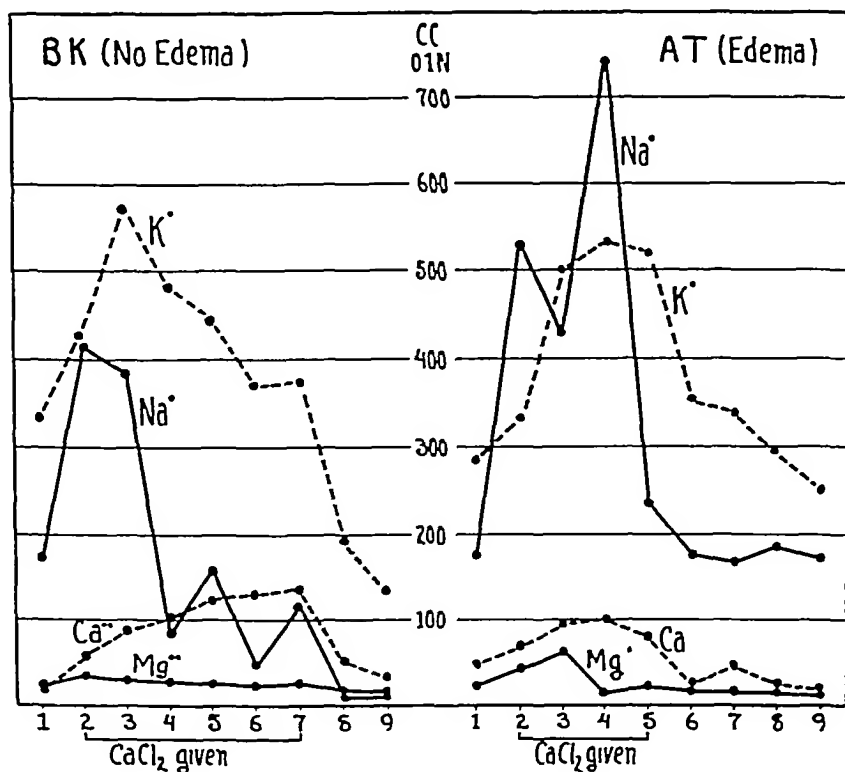


FIG 9 DATA FROM B K (NO EDEMA) AND A T (EDEMA) SHOWING COMPOSITION OF FIXED BASE EXCRETION IN URINE DURING 4-DAY PERIODS OF  $\text{CaCl}_2$  ADMINISTRATION

Measurements from 24-hour urine specimens

intra- and extra-cellular compartments, from the former more rapidly at first and from the latter over a longer period. A glance at the diagrams also provides the information that in the case of B K. (no edema) about the same amounts of extra- and of intra-cellular water have been withdrawn whereas the much larger water loss from

A T (edema) is, as would be expected, largely composed of extra-cellular water. The data in table 5 will serve to illustrate further these differences as regards amount and source of the withdrawn water depending on the presence or absence of edema fluid in the body. The values given are for total increase of excretion of each of the four bases for the first four days and for the entire eight days of the study periods computed from the fore period values. Here may be noted the much larger excretion of Na than of K during the period of  $\text{CaCl}_2$  administration in the case of A T (edema) and also the lack of recovery of Na during the four-day after period. In contrast, B K loses rather more of K than of Na during the first four days and entirely replaces Na during the following four days although  $\text{CaCl}_2$  ingestion

TABLE 5

*Composition of increase of fixed base excretion in urine produced by  $\text{CaCl}_2$*

Period		Total fixed base	Na	K	Ca	Mg
days		cc 0.1 N	cc 0.1 N	cc 0.1 N	cc 0.1 N	cc 0.1 N
4	B K (no edema)	1242	455	569	284	37
	A T (edema)	2200	1238	751	157	54
8	B K (no edema)	688	-178*	296	553	29
	A T (edema)	2212	1245	859	81	27

\* The minus sign here indicates that according to the rough method of obtaining this value the earlier loss of Na was to this extent more than replaced.

was continued through the first two of these days. A point of interest appearing in this table is the large total (553 cc 0.1 N) for increase in Ca excretion for the eight-day period of B K (no edema). This value constitutes nearly all of the base deficit (688 cc 0.1 N) remaining at the end of the period. In the case of A T (edema) the much larger base deficit (2212 cc 0.1 N) contains a relatively very small amount (81 cc 0.1 N) of Ca. That Ca may be withdrawn from Ca deposits for use in the process of acid excretion in defense of the fixed base concentrations of the body fluids has been shown by Goto in the case of animals fed with mineral acids. Gamble, Ross and Tisdall found evidence that Ca is used in this way during the acidosis produced by fasting. Here Ca is abundantly available from the ingested salt and it is interesting to find that in the case of a correct total fixed base

content in the body (B K) Ca seems to be used defensively whereas in the presence of superfluous fixed base (and water) the excretion of Ca in the urine is apparently so managed as not to appreciably obstruct a removal of Na and K

## VII DISCUSSION

In undertaking to explain the diuretic action of acid producing salts in terms of the findings given above, we unfortunately at once encounter a familiar dilemma. Is the removal of body water a consequence of a forced withdrawal of fixed base or, vice versa, is a reduction of the volume of body water the initial event? We may fairly reasonably imagine that the huge addition to the excess of acid over fixed base presenting for excretion in the urine when these salts are given overtaxes the factors defending fixed base in the body fluids and produces a loss of fixed base which is accompanied by the water which contained it within the body. If this be the case, and regarding the kidney as the locus of the regulated production of ammonia, as has been well argued by Nash and Benedict (16), the much larger removal of fixed base obtained in the presence of nephritis may be plausibly explained by assuming that advantage is here taken of a disabled ammonia factor. Such an explanation excellently suits the relationship of the three factors as displayed in the diagrams in Section V, but does not well agree with the fact that non-nephritic edema may sometimes be effectively treated by ingestion of acid producing salts.

The alternative view of the sequence of events following ingestion of the acid producing salts consists in surmising a reduction in the volume of body water caused by the demonstrated increase in acidity and possibly other physico-chemical changes. The volume of body water being set at lower level in consequence of alteration of internal factors controlling it, the mechanism regulating the excretion of fixed base correctly permits the cast out body water to carry with it its content of base. A causative relationship of an increased acidity in the body fluids to secretion of a portion of body water may not at present be assumed with the certainty that applies in referring the increased fixed base excretion to the deficit in ammonia production. We have, however, the knowledge that an increased acidity in the body fluids



lowers the base binding capacity of protein, a change which must according to the terms of the Donnan equilibrium theory considerably alter osmotic pressure adjustments between the several compartments of body water. According to J. B. S. Haldane (9), the changes produced by an increased acidity should favor a movement of water in the direction of the kidney. The view that the diuretic action of these acid producing salts is in some way a consequence of the increased acidity which they produce in the body fluids is obviously the preferable one since it includes in the mechanism of this action the striking alterations of structural factors found in the blood plasma and also permits a quite satisfactory explanation of the increased excretion of fixed base in the urine.

#### VIII SUMMARY

Following ingestion of  $\text{CaCl}_2$ ,  $\text{NH}_4\text{Cl}$ ,  $\text{MgSO}_4$  or of  $(\text{NH}_4)_2\text{SO}_4$  in considerable amounts, the quantity of inorganic acid radicals which must be conveyed through the body fluids is greatly increased without an appreciable accompanying increase in the amount of fixed base presenting for transport. These salts are for this reason described as acid producing.

Administration of these acid producing salts causes an increased acidity of the body fluids demonstrable in the blood plasma and an increased excretion of fixed base in the urine. The immediate cause of the increased acidity of the body fluids is an extension of  $(\text{Cl}')$  with the result that, fixed base remaining fairly stationary ( $\text{BHCO}_3$ ) is equivalently reduced, a change which is only slightly compensated for by respiratory adjustment of  $(\text{H}_2\text{CO}_3)$ . This increase of  $(\text{Cl}')$  is found following administration of the sulphates as well as after ingestion of the chlorides. In the presence of edema and nephritis an acidosis due to extension of  $(\text{Cl}')$  was found to be already established before the acid producing salts were given. Probably in consequence, the degree of increased plasma acidity found after administration of the salts was much greater than in the case of subjects without edema and having an initially usual plasma  $(\text{Cl}')$ . The increased excretion of fixed base in the urine represents a deficit in the increase of ammonia production as compared with the increase in acid excretion caused by the ingested salt and was found to be much larger and longer sustained in the presence of edema.

The changes which the acid producing salts cause in the blood plasma are accompanied by a removal of body water which, as indicated by the nearly stationary values for fixed base found in the plasma, is closely proportional to the increase of fixed base excretion in the urine. Regarding the composition of the increase in fixed base excretion as indicating the source of the withdrawn body water, an analysis of this increase shows a withdrawal of both extra- and of intra-cellular water, of the former at first more rapidly and of the latter over a longer period. In the presence of edema much the greater part of the water removed is from the extra-cellular compartment.

The diuretic action of these salts may probably be correctly referred to the increase in acidity of the body fluids which they all, as regards immediate cause, produce in an identical manner. The change in reaction is of such degree that it must according to the terms of the Donnan equilibrium theory considerably alter osmotic pressure values in the body fluids, and these alterations may reasonably be suspected as the factors in the ensuing removal of a portion of body water. In the presence of a reduction of the volume of body water, the increased excretion of fixed base in the urine is easily understandable as a correct adjustment of the mechanism controlling fixed base excretion in defense of a stationary concentration within the body.

#### CHEMICAL METHODS USED

##### *Blood plasma values*

These, except for pH, were actually obtained in samples of blood *serum* for the reason that the total fixed base measurement obviously could not be obtained in oxalated or citrated samples. "Plasma" being for purposes of discussion the more suitable term, is used throughout and the probably very slight error contained in the assumption that plasma values may be measured in serum samples is neglected. The blood samples were collected in a Luer glass syringe and then delivered through small bore glass tubing and under oil into 15 cc. centrifuge tubes which were filled to the top and stoppered with a one-hole rubber stopper, permitting expulsion of superfluous oil, and the hole then closed with a glass plug. References to descriptions of the methods used are as follows: *Total Fixed Base*, Fiske (17), *Bicarbonate*, Van Slyke (18), *Chlorides*, Fiske (to be published), *Phosphates (inorganic)*, Briggs (19), *Sulphates (inorganic)*, Denis (20), *Calcium*, Kramer and Tisdall (21), with the alteration that, instead of using the permanganate titration, the ppt. was ignited in a platinum dish then dissolved in 0.015 N HCl and titrated back with 0.01N NaOH (suggested by Fiske). pH was determined in

separately obtained samples by a modification of Cullen's colorimetric method devised by Hawkins (22)

### *Urine values*

*Total Fixed Base*, Fiske (17), *Chlorides*, Volhard titration, *Phosphates (inorganic)*, Briggs (19), *Sulphates (inorganic)*, Fiske (23), *Ammonia*, Folin and MacCallum (24), *Potassium*, Tisdall and Kramer (25), *Calcium*, some of the measurements were obtained by the method of McCrudden (26) and others by the micro-method of Tisdall and Kramer (25), *Magnesium*, McCrudden (26) and also Briggs (27), *pH*, Palmer and Henderson (28) Toluol was the preservative used during collection of the 24-hour specimens

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# CLINICAL OBSERVATIONS ON THE T WAVE OF THE AURICLE APPEARING IN THE HUMAN ELECTROCARDIOGRAM

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There has been considerable difference of opinion during the development of electrocardiography as to the relationship between the electrical phenomena of the heart beat, as recorded by the electrocardiograph, and the attendant muscular activity. It has come to be generally held that the waves of the electrocardiogram indicate the spread of relative electrical negativity throughout the heart from the region of the sino-auricular node to the various parts of the heart's musculature. The electrical energy propagated in this manner has been the expression of the so-called "excitation wave."

Much physiological research has been undertaken in an attempt to demonstrate the time relationship between this "excitation wave" and the muscular contraction of the heart. In general most of such work has been by the method of simultaneous registration of electrocardiographic and myocardiographic records in such a manner that they can be compared, and the sequence of events studied and measured. Similar comparisons have been made between electrocardiograms and intracardiac pressure curves. Myocardiographs have been devised by Cushny, Roy, Gesell, Wiggers and others, and have been used in connection with the string galvanometer, for which we are indebted to Professor W. Einthoven.

By the extensive researches of Garten and Weber, Wiggers, Lewis, Feil and Stroud, Wiggers and Dean, and others, it became generally recognized that the "excitation wave" precedes the muscular contraction of the heart by a definite and constant interval. Wiggers (1)

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says "comparison of the  $P_2$  wave with the intra-auricular pressure curves indicates that auricular systole begins near the S-A node about 0.02 second after the rise of  $P_2$  (Garten and Weber, Wiggers), i.e., on its ascending limb. Not until 0.04 second later, however, or until the entire P wave has been completed, has the tissue near the right auricular appendage even begun to contract." Similarly "as regards the relation of the R wave to the onset of ventricular systole, most of the earlier experimental evidence favors the idea that the  $R_2$  variation is practically completed before mechanical systole has begun." Wiggers found that "the onset of the (intraventricular) pressure rise is uniformly somewhat later, that is, 0.03 to 0.045 second after the initial rise of the  $R_2$  wave. It is significant, therefore, that the  $R_2$  wave precedes by a short though definite interval the first evidence of mechanical activity in the ventricle." So far as the T wave was concerned he concluded that "it is quite evident that the end of ventricular systole cannot be definitely related to any phase of the  $T_2$  variations."

So firmly had the idea of an electrical wave spreading throughout the heart in advance of mechanical response been fixed in the theory of cardiac activity that it seemed one of the axioms of physiology.

In October, 1924, Professor Einthoven delivered at the Harvard Medical School the Dunham Lectures. His subject was "The Relation of the Mechanical and Electrical Phenomena of Muscular Contraction with Especial Reference to Cardiac Musculature." In these lectures he presented evidence to prove that the electrical phenomena of both skeletal and cardiac muscle do not precede the mechanical activity but are in every case absolutely coincident, and also that in force as well the electrical and mechanical phenomena are uniform. He maintained that with the apparatus used by previous investigators it was impossible for myograms recorded by them to show either the initial activity or minimal extent of muscular contraction. In particular he disagreed with previous findings showing a latent period between the "excitation wave" and the muscular response in skeletal and cardiac muscle, he also showed that observations on dying hearts which were said to show electrical activity after cessation of mechanical action were incorrect.

In proving his view he described an apparatus which he had devised

whereby photographic records of muscular action could be made by means of a frictionless lever and high magnification. Simultaneous records obtained with this apparatus and with the string galvanometer demonstrated the precise coincidence of electrical and muscular activity. In experiments on artificially poisoned hearts he was able to show not only that electrical and muscular phenomena begin and stop at the same instant, but that the amplitudes of the curves in hearts that are slowly dying or undergoing recovery are perfectly comparable in magnitude. In other words, diminution of muscular activity is accompanied by a corresponding diminution in the size of the electrical complexes, and increase in contractile power, as measured in the myocardiogram, is perfectly reflected in an increased amplitude of the electrocardiographic waves.

The application of this work to human cardiology affords further important proof of Einthoven's thesis. This is connected with a study of the P wave of the electrocardiogram. In the early work in electrocardiography it was thought to be improbable that the relatively long activity of auricular systole could be accurately represented by such a momentary phenomenon as the P wave of the electrocardiogram. Measurements readily showed that the duration of the contraction of auricular muscle was far greater than the time expressed by the P deflection. It would, therefore, seem difficult to reconcile the muscular and electrical activities in the case of the auricle—a situation more easily capable of explanation in the ventricular records because of the existence of the T wave, which may be shown to have a relationship with the end of ventricular systole.

Einthoven, however, has brought forward some important observations bearing on this subject. He pointed out that H. E. Hering (2) had noted in 1908 that under certain circumstances it was possible to demonstrate that the auricle also had a T wave associated with its contraction. Hering found this wave in electrocardiograms from a frog's heart. He also observed a similar wave in records from a dog's heart in which the auricles only were beating, as the ventricles were in fibrillation from the action of curare. Other workers, Straub (3), Henle (4), and Eger (5), had previously published electrocardiograms from frogs showing such curves, which they were unable to explain. Noyons (6) in 1910 had also made similar observations on the cold-blooded heart.

Experiments on frogs' hearts by Samojloff (7) in 1908 had revealed a wave which he called the B wave, as he attributed it to muscular activity in the bulbus aortae. Hering, in repeating these experiments, cut away the bulb and found that this wave continued to be recorded. He therefore concluded that it did not arise in the bulb. By producing premature ventricular contractions, Hering was able to make this wave fall upon the R wave and also to follow the R wave, but again observed that its relationship to P and its form were not influenced by ventricular activity. He therefore came to the conclusion that it represented a T wave of the auricle, and suggested naming it the "Ta" wave.

Bakker (9) in 1912 demonstrated an auricular T wave in the eel's heart. In the same year Fredericq (8) published an article on the nature of auricular systole, with electrocardiograms from dogs' hearts, showing that the electrical record from the auricle was essentially the same as that from the ventricle and consisted in an early rapid deflection followed by a curve which was much slower in its rise and fall.

Eiger (10) in 1913 added greatly to this study by an article on the electrocardiogram in which he showed that not only the ventricle and auricle have T waves connected with their activity but also that the bulbus aortae and sinus venosus have such waves. Veen (11) the following year made a further contribution and suggested a different nomenclature for the waves.

Eyster and Meek (12) in 1913 published electrocardiograms showing auricular T waves in records from the tortoise heart in which the ventricle had ceased to beat as the result of the application of a second Stannius ligature. In addition they found such waves in electrograms taken from isolated strips of ventricular muscle. They also demonstrated auricular T waves in electrocardiograms from dogs' hearts in which there was heart block from morphin, from pressure in the region of the auriculo-ventricular node, and from vagus stimulation.<sup>1</sup>

Rumke (13) in 1916 summarized this literature and again emphasized the viewpoint that isolated parts of the heart show electro-

<sup>1</sup> J. Meakins (Heart, 1913-1914, v, 287 and 288) also published electrocardiograms from dogs showing "Sa" waves. Figs 4, 7 and 8.

grams in which initial deflections are followed by T waves, and that leads from any two parts of the heart will record such combined deflections as the inherent phenomenon of muscular contraction

The presence of an auricular T wave in the human electrocardiogram has been, of course, suspected, but under normal circumstances it can not be demonstrated, due to the fact that the Ta wave in such cases occurs at the same time as the QRS complex and its relatively low potential has no appreciable influence on the form of the ventricular wave. Theoretically, therefore, it would be possible to demonstrate the Ta wave only where one could isolate the auricular complex in its entirety from the ventricular response. Such a condition exists in heart block and is most favorably shown in complete block in which it is possible to see P waves occurring as isolated complexes uninfluenced by the contiguity of QRS groups.

The presence of auricular T waves in cases of heart block has long been known and has recently been described by a Japanese worker.<sup>2</sup> Einthoven has found in the discovery of the auricular T wave another instance of electrical activity paralleling muscular contraction. He has been able to relate it to the terminal phase of auricular systole in the same manner that the ventricular T wave is related to ventricular activity. It is the clinical electrocardiographic demonstration of this Ta wave in cases of heart block that we wish to note in this observation.

#### ELECTROCARDIOGRAPHIC OBSERVATIONS

In the past 10 years in the Cardiac Clinic of the Massachusetts General Hospital, 37 cases of complete heart block have come under observation, and have been studied by electrocardiograms. It was decided to review the records of these patients in an attempt to discover whether or not any of them showed the presence of Ta waves. Certain difficulties in the determination of these waves were immediately obvious. (1) the records must be technically accurate, free from any marked somatic tremor or extraneous deflections, (2) they must show solitary auricular complexes sufficiently isolated as to be

<sup>2</sup> Personal communication from Prof. Einthoven. We have been unable to find this reference. Auricular T waves have also been described in the human electrocardiogram by Boden and Neukirch (18) and by Boden (19).



uninfluenced by any QRS or ventricular T deflections, and, (3) the preceding conditions must occur in cases in which the Ta wave is not isoelectric, as we must suppose it sometimes is, in analogy with the ventricular T wave

In this series of 37 cases of complete heart block we were able to demonstrate a Ta wave in 18 instances, and in 7 more a questionable Ta wave could be seen. In addition it was clearly shown in one case of high grade partial block, with a 3:1 ratio of auricular and ventricular beats

Table 1 shows the amplitude of the Ta deflections in general terms

The Ta wave is of very low potential and in none of these cases does the deflection exceed 2 mm in height with normal tension of the electrocardiographic string (standard of 1 millimeter excursion for each tenth of a millivolt of potential). However, in this series

TABLE 1

*Amplitude of Ta waves in series of 37 heart block cases*

	No Ta wave demonstrable	Questionable Ta wave	Slight diphasic or inverted Ta wave	Moderate Ta wave	Marked Ta wave
Number of cases	12	7	6	8	4

approximately one-half of the cases show waves following the P waves at constant intervals which must be interpreted as being the T deflections of the auricular complexes

The initial deviation of the Ta wave in these cases was constantly negative, i.e., in the opposite direction to the P wave, and the whole complex seemed typically to be represented as a diphasic P wave in which the return to the base line was gradual. In the most marked cases a very short isoelectric period separates the P and Ta waves

Simple measurements, without the use of a comparator, show that the Ta wave varies in length from 0.22 to 0.26 of a second. The total duration of the auricular complexes from the beginning of the P wave to the end of the Ta wave varies from 0.34 to 0.42 of a second. Measurements from the electrocardiograms of the cases in which the Ta wave was most obvious appear in table 2

It will be seen from this table that the average total length of the

auricular complexes is about equal to three and a half times the average length of the P wave. It is also evident that the average Ta wave differs from the average T wave of the ventricle in deviating from the base line earlier after the end of the P wave than does the T after the QRS. The isoelectric period, therefore, is very short (0.01 to 0.05 of a second) and often is almost indistinguishable.

TABLE 2

Case	Length of P wave	Length of isoelectric period between P and Ta	Length of Ta wave	Total length of auricular complexes
	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>
I	0.12	0.04	0.26	0.42
II	0.08	0.05	0.23	0.36
III	0.11	0.02	0.23	0.36
IV	0.10	0.01	0.25	0.36
IV	0.10	0.02	0.22	0.34
V	0.13	0.03	0.22	0.38
Average	0.106	0.03—	0.23+	0.37

TABLE 3

*Comparison of electrocardiograms and myograms taken simultaneously from experimental animals*

Author	Experimental animal	Length of P wave	Latent period of auricular muscle	Length of auricular myogram
		<i>seconds</i>	<i>seconds</i>	<i>seconds</i>
Lewis, Meakins, and White (14)	Dog	0.06	0.03	0.16
Lewis (15)	Dog	0.04	0.015	0.16
Lewis and White (16)	Dog	0.06	0.03	0.14
Kraus and Nicolai (17)	Dog	0.08	0.10	0.20
Average		0.06	0.044	0.16+

In order to establish a relationship between the length of the total auricular complex and the muscular activity of the auricle it was decided to study the relative length of the P wave in experimental animals compared to the myocardiographic records of the auricles.

If the P-Ta complex were an accurate measure of the muscular contraction one might expect to find that the length of the P wave was about one-third of the total length of the auricular myogram added

to the latent period of the muscle. As the P wave is quite variable in length this ratio can only be an approximation. Comparative measurements from electrocardiograms and myograms taken by various workers appear in table 3.

From these figures it appears that the ratio is roughly what one would expect, the P wave in each instance having a duration of about one third to one quarter of the auricular myogram plus its latent period.<sup>3</sup> This is further evidence that the P-Ta complex is an exact measure of auricular muscle activity.

The cases of our series in which the Ta wave is most marked are summarized below, and are illustrated by electrocardiograms showing the characteristic appearance of the Ta wave.

*Case 1* D H D Garage Manager Age 53 First seen April 13, 1917, when he was admitted to the Massachusetts General Hospital complaining of syncopal attacks for 8 months associated with a pulse rate of 35. There was a very vague history of syphilis and the blood Wassermann was negative. Electrocardiogram showed complete heart block with ectopic ventricular contractions. The ventricular rate sometimes dropped to 24. A diagnosis of heart block with Stokes-Adams attacks, probably arteriosclerotic in origin, was made. He died in 1919.

*Case 2* C A P Salesman Age 45 Seen first November 30, 1916, complaining of fatigue for 4 months, with attacks of faintness without loss of consciousness. His pulse suddenly dropped to 26 during an attack. Electrocardiogram showed partial and complete A-V block, in one instance with eleven auricular beats without one ventricular response. There was a suspicious history of syphilis and the blood Wassermann was moderately positive. A diagnosis of heart block, probably of luetic origin, with Stokes-Adams syndrome, was made. He died in April, 1917.

*Case 3* J E R Shipping clerk Age 52 Seen first February 9, 1922, with a complaint of dyspnea on exertion for a few months and sudden attacks of syncope. His pulse varied from 20 to 45 and auricular sounds were clearly audible. Electrocardiogram showed high grade partial A-V block, 2:1 and 3:1, and at times complete dissociation. A diagnosis of arteriosclerotic heart disease with heart block and Stokes-Adams attacks was made. He died suddenly in November, 1923.

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<sup>3</sup> The ratio of the average length of the P wave to the average length of the auricular complex in the human electrocardiogram, in the cases measured, is 1:3.49. The ratio of the P wave to the auricular myogram, in the case of the experimental animals, is 1:3.4. As the series in both instances is so small such close agreement cannot be considered as necessarily constant.

*Case 4* P S Girl First seen by Dr Edwin H Place in 1907, aged 2-3 years, with tonsillar diphtheria. There was a rather indefinite story of congenital heart disease which was not demonstrable when she was first observed. On the third day of her diphtheria her pulse suddenly dropped from 120 to 50. She recovered from her infection but the slow rate has persisted ever since, never going above 50. Electrocardiographic study in 1916 and 1919 showed complete A-V block. When she was last seen in the Spring of 1924 she was feeling perfectly well. There is doubt as to the actual causation of this permanent block by the diphtheria in the absence of other evidence of serious myocardial damage or peripheral paralysis.

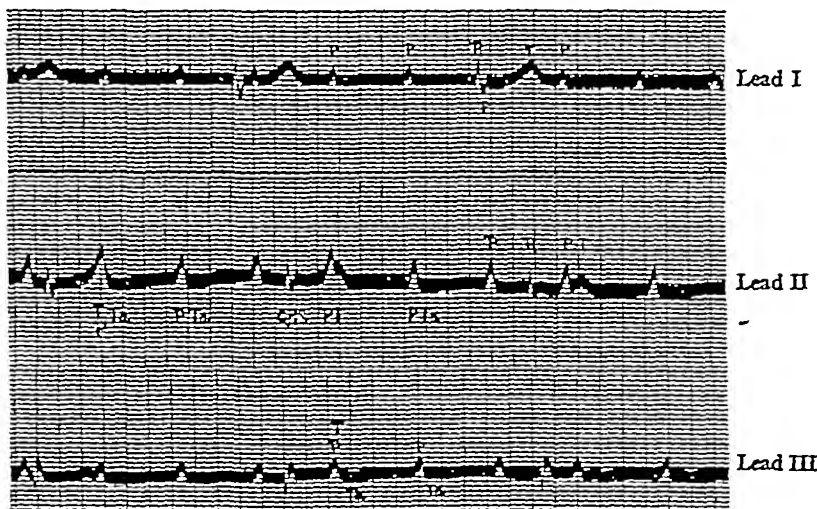


FIG 1 (CASE 1) CLEAREST Ta WAVES IN LEAD II, LESS DISTINCT IN LEAD III, ALMOST ISOELECTRIC IN LEAD I

*Case 5* E D G Student Age 24 First seen January 5, 1924, when he was admitted to the Massachusetts General Hospital with a cellulitis of the neck and osteomyelitis of the jaw following the extraction of abscessed teeth. He developed precordial pain and friction rub, and it was thought that he had acute pericarditis and mediastinitis. During the acute period of his illness his pulse suddenly dropped from 88 to 40, and electrocardiograms showed high grade partial block (3, 4, and 5 to 1 ratio of auricular and ventricular beats). At times sounds suggestive of auricular contractions were heard. The heart block disappeared in about 5 days and he recovered. His heart showed no signs of endocarditis and there was no pericardial effusion. A diagnosis of toxic or bacterial myocarditis with heart block was made.



FIG 2 (CASE 1) WELL MARKED Ta WAVES THROUGHOUT LEAD II HIGH P WAVES

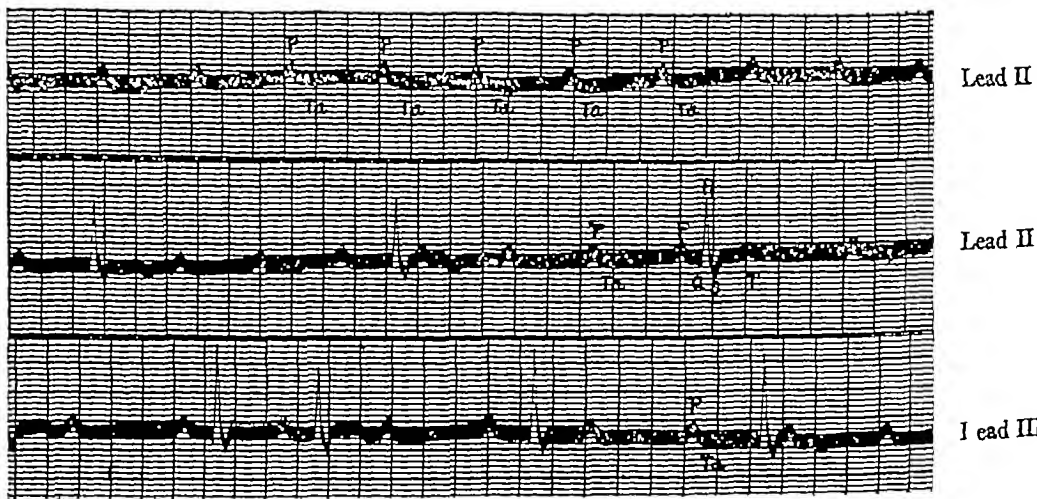


FIG 3 (CASE 2) ELEVEN AURICULAR WAVES IN LEAD II WITH NO VENTRICULAR RESPONSE Ta WAVES WELL MARKED IN ALL LEADS

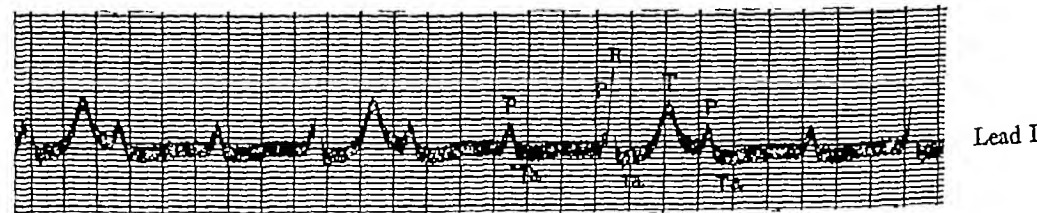


FIG 4 (CASE 3) DISTINCT Ta WAVES, EVERY THIRD ONE BEING PARTLY OBSCURED BY THE VENTRICULAR COMPLEXES

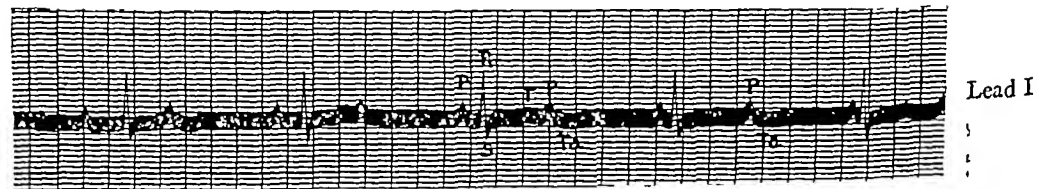


FIG. 5. (CASE 4.) WELL MARKED Ta WAVES, AT TIMES OBSCURED BY VENTRICULAR COMPLEXES





## SUMMARY

1 The generally recognized opinion concerning cardiac mechanism includes the theory of an electrical "wave of excitation" spreading throughout the heart by way of the conducting system, slightly in advance of the attendant muscular contraction. This theory is based on simultaneously recorded myocardiographic and electrocardiographic waves.

2 Professor W. Einthoven has recently denied the existence of this latent period and has described apparatus by which he has been able to record electrocardiograms and myocardiograms that show that electrical and muscular activity are precisely coincident. Previous observations, he asserts, are faulty because of the inadequacy of the apparatus used.

3 In support of his position Einthoven quotes the discovery of Henning and others that the auricular complex of both cold and warm-blooded animals has a terminal deflection, or T wave, which Henning called the Ta wave.

4 We have been able to find a Ta wave occurring after the P wave in electrocardiograms of clinical cases showing complete or high grade partial A-V block. This wave was found in 18 out of 37 cases of complete block seen at the Massachusetts General Hospital.

5 The duration of the P wave in the human electrocardiogram is about one third of the total duration of the P-Ta complex. This is consistent with the findings in experimental animals that the P wave is approximately one third to one quarter as long as the auricular myogram plus its "latent period" and supports the view that the Ta wave is an accurate expression of the terminal phase of auricular muscle activity.

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# THE FACTORS IN THE DEHYDRATION FOLLOWING PYLORIC OBSTRUCTION

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## INTRODUCTION

The data presented in this paper were obtained from several dogs following experimental obstruction of the pylorus. The experiments were undertaken with the purpose of learning the chief factors in the causation of the rapid dehydration which follows pyloric obstruction and of explaining the prevention or repair of this change which is obtained by introducing sodium chloride and water into the body.

In undertaking to investigate the cause of a reduction of the volume of body water it should at once be admitted that we have as yet only a fragmentary knowledge of the many parts of the regulatory mechanism which under usual circumstances accomplishes the remarkable adjustment of maintaining, in several elastic compartments, a closely stationary total volume of water. Certain gross data are, however, at hand which fairly satisfactorily serve the purposes of this study. It has been shown that in the presence of considerable reductions of the volume of body water the total concentration of dissolved electrolytes tends to remain stationary (1, 2). It is therefore probably permissible to postulate a close dependence of the volume of body water on the total quantity of dissolved electrolytes which the body contains. In this study the premise is used that a withdrawal of the electrolytes of the body fluids will be accompanied by a proportionate reduction of the volume of body water and that this change can only be repaired by replacing both the lost water and the lost electrolytes.

The relative structural importance of each of the electrolytes in blood plasma is indicated by the diagram in figure 1, which presents

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their average normal concentrations in terms of acid-base equivalence<sup>2</sup> As may be seen Na constitutes nearly all of the plasma base and Cl' is the chief factor on the acid side The acid-base balance of this structure is preserved by the immediate and automatic adjust-

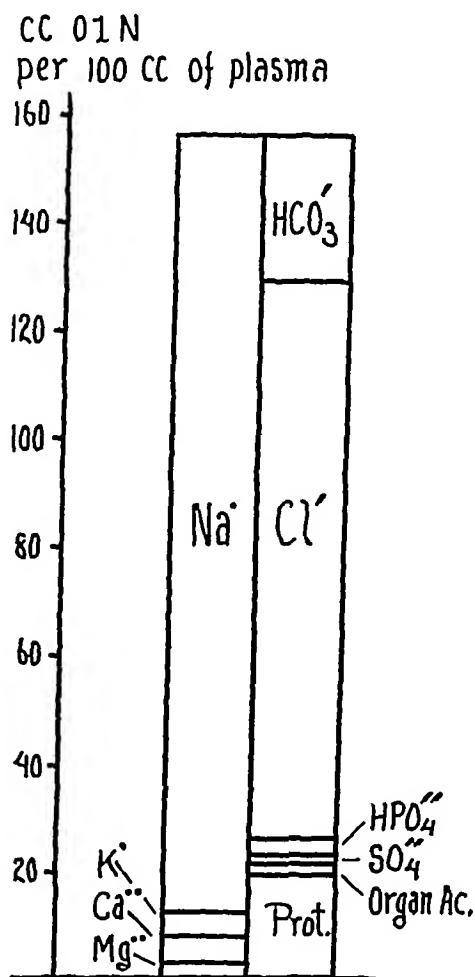


FIG 1 REPRESENTING THE STRUCTURAL FACTORS OF NORMAL HUMAN BLOOD PLASMA IN TERMS OF ACID-BASE EQUIVALENCE AT PH 7.4

The factors are superimposed, those of base in the left hand, and those of acid in the right hand column

<sup>2</sup> In this diagram the plasma salts are represented as completely dissociated. In order to simplify discussion of the interrelationship of the structural factors in the plasma, the presence of relatively very small concentrations of molecules is ignored and the plasma structure is regarded as composed of separately determined concentrations of ions.

ment of ( $\text{HCO}'_3$ ) in the presence of a change in any of the other factors. Owing to this capacity of ( $\text{HCO}'_3$ ) for varying readily the total ionic concentration in the plasma, is determined by the concentration of the base. From these statements it may be deduced that a withdrawal of  $\text{Cl}'$  from the plasma will be replaced by an increase of ( $\text{HCO}'_3$ ) and will therefore not cause a reduction of the total concentration of ions. A loss of Na however is not so readily replaceable and moreover also involves a loss of its equivalence of  $\text{HCO}'_3$ . Loss of  $\text{Cl}'$  thus does not deplete the ionic content of the plasma, whereas loss of Na does and doubly. This point is presented here for the reason that a chief purpose of this study is to demonstrate that a loss of Na from the body is the essential factor in the rapid dehydration which follows obstruction of the pylorus.

TABLE 1  
*Measurements from blood serum samples, experiment I*

Number	Taken	Chloride	Bicarbonate	Urea	Protein
		grams NaCl per liter	vol per cent $\text{CO}_2$	mg per 100 cc.	per cent
1	Before operation	6.13	51.9	19	7.9
2	18 hours after operation	5.40	82.5	15	9.6
3	29 hours after operation	4.73	82.4	24	9.9
4	42 hours after operation	3.93	72.1	54	11.3

Following pyloric closure, ( $\text{Cl}'$ ) in the plasma is reduced to a greater extent than ( $\text{Na}$ ). If these defects in the ionic structure are correctly repaired by the introduction of NaCl into the body, more of  $\text{Cl}'$  than of Na must be retained. Data were obtained from urine specimens collected during a period of NaCl administration which illustrate the adjustments used in defense of ( $\text{Cl}'$ ) in the body fluids in the presence of an excess of Na presenting for excretion in urine.

## EXPERIMENTAL

### *Experiment I*

Protocol Male dog Weight 17.3 kilos Under ether anesthesia the stomach was severed just above pylorus and closed by means of a purse string suture. The proximal end of the duodenum was closed in the same way. The abdominal wound was strongly stitched and protected by means of a gauze and collodion dressing. The dog was permitted to drink water freely. Moderate but definite muscular

twitchings were observed beginning about 16 hours after the operation. No convulsions were seen. The animal became very dull and feeble about 40 hours after the operation, and 10 hours later was found dead.

In table 1 are given measurements of bicarbonate, chloride, urea, and protein in samples of blood serum obtained before and at intervals following the operation. The measurements illustrate the changes which have been found (3, 4, 5) to follow characteristically experimental closure of the pylorus. As may be seen, there occurs a large reduction of plasma chloride, an event readily explained as due to a withdrawal of  $\text{Cl}'$  from the body in consequence of a continued vomiting of gastric secretions. The fall in plasma chloride it will be noted is accompanied by a rise in bicarbonate. Another outstanding change is a rapid and extensive dehydration of the plasma indicated by a progressive increase in the concentration of protein. A moderate impairment of renal function shown by a rise in the urea content of the plasma may reasonably be referred to the lack of water intake, there being practically no absorption of the water taken into the stomach.

TABLE 2

*Measurements of chloride and bicarbonate given in table 1 compared in terms of tenth normal solutions*

	Sample No 1	Sample No 2	Sample No 3	Sample No 4
$\text{BCl}$ , cc 0.1 N per 100 cc	106	93	82	68
$\text{BHCO}_3$ , cc 0.1 N per 100 cc	23	37	37	32
$\text{BCl} + \text{BHCO}_3$ , 0.1 N per 100 cc	129	130	119	100

A relationship of the chloride reduction and bicarbonate increase is shown by placing in table 2 the measurements of these two values expressed in equivalent terms, viz., as cubic centimeter of tenth normal solutions per 100 cc of plasma. If the concentration of fixed base in the plasma remains stationary a reduction of ( $\text{Cl}'$ ) may be expected to produce an equivalent extension of ( $\text{HCO}_3'$ ). That is, base released by  $\text{Cl}'$  will be immediately balanced by  $\text{HCO}_3'$ , derived from the free carbonic acid in the plasma and this in turn replenished from the  $\text{CO}_2$  production in the body. As may be seen in the table, ( $\text{Cl}'$ ) + ( $\text{HCO}_3'$ ) is almost the same before and 18 hours after operation. However, 29

hours and 42 hours after closure of the pylorus ( $\text{Cl}'$ ) + ( $\text{HCO}_3'$ ) is found to be less than the initial sum of these values, indicating that extension of ( $\text{HCO}_3'$ ) is less than the lowering of ( $\text{Cl}'$ ) This lack of full replacement of  $\text{Cl}'$  by  $\text{HCO}_3'$  suggests a loss of fixed base from the plasma That this occurs following experimental obstruction of the pylorus has been thoroughly shown by Hastings, Murray and Murray (6) and in our subsequent experiments

### *Experiment II*

**Protocol** Small male dog Weight before operation and after 12 hours of fasting 5.71 kilos Abdomen opened under ether anesthesia and pylorus closed by ligature Wound closed and protected as in first experiment Five injections of sterile and warmed 5 per cent glucose solution into the peritoneal cavity, each of 250 cc., were given 16, 27, 40, 51 and 62 hours after operation Blood samples were taken from 4 to 7 hours after glucose injections The dog became rapidly weak and apathetic during the early part of the third day of the experiment, a few hours later began to have convulsions and from then until death 72 hours after operation was almost constantly in a state of clonic rigidity Weight after death 5.20 kilos

The measurements of plasma values obtained during this experiment are given in table 3 Those of protein indicate marked dehydration of the plasma, though apparently not so extensive as in experiment I Comparing the chloride values in tables 3 and 1, the rate of reduction is seen to be approximately the same Table 3 also contains measurements of sodium and these show a progressive loss of this structurally most important electrolyte of the plasma In spite of 1250 cc of water administered to this animal weighing only 5710 grams, there occurred during three days a loss in body weight of 500 grams These data will serve to illustrate the inefficacy of water alone to sustain the usual volume of the body fluids in the presence of a continued withdrawal of their chief structural components It has been thoroughly shown by Haden and Orr (7) that the survival period in dogs, following pyloric or upper intestinal obstruction, cannot be appreciably if at all prolonged by subcutaneous injections of distilled water or of glucose solutions

An analysis of the relationship of the changes in ( $\text{Cl}'$ ), ( $\text{HCO}_3'$ ) and ( $\text{Na}$ ) is given in table 4, the measurements in Table 3 being here presented as cubic centimeters 0.1 N per 100 cc of plasma The pro-

gressive decrease in the values for  $(\text{Cl}') + (\text{HCO}_3')$  shows the diminution of chloride to be increasingly greater than the accompanying increase of bicarbonate. It was suggested above (experiment I) that this incomplete replacement of loss of  $(\text{Cl}')$  by increase of  $(\text{HCO}_3')$  is probably due to a depletion of plasma base. The measurements of  $(\text{Na})$ , which constitutes more than 90 per cent of the total fixed base in blood

TABLE 3  
*Measurements from blood serum samples, experiment II*

Number	Taken	Chloride	Bicarbo- nate	Sodium	Phos- phorus	Protein
		grams NaCl per liter	vol per cent $\text{CO}_2$	mg per 100 cc	mg per 100 cc	per cent
1	Before operation	6.53	58.9	337	7	6.9
2	23 hours after operation	5.07	60.7	315		8.6
3	45 hours after operation	3.80	73.9	294	12	8.8
4*	66 hours after operation	2.40	80.7	281	14	8.2

\* Ketone acids, 0.28 gram (as B-oxylutynic) per liter

TABLE 4  
*Measurements of chloride, bicarbonate and sodium given in table 3 compared in terms of tenth normal solutions*

	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4
$\text{BCl}$ , cc 0.1 N per 100 cc	113	87	66	41
$\text{BHCO}_3$ , cc 0.1 N per 100 cc	26	27	33	36
$\text{BCl} + \text{BHCO}_3$ , cc 0.1 N per 100 cc	139	114	99	77
$\text{Na}$ , cc 0.1 N per 100 cc	146	137	128	122
$\text{BCl} + \text{BHCO}_3$ , cc 0.1 N per 100 cc	139	114	99	77
$\text{Na} - (\text{BCl} + \text{BHCO}_3)$ , cc 0.1 N per 100 cc	7	23	29	45

plasma, show a progressive reduction in its concentration. Subtracting, however, the values for  $(\text{Cl}') + (\text{HCO}_3')$  from those for  $(\text{Na})$ , remainders are obtained which are progressively larger (see table 3). It is thus apparent that besides a loss of sodium from the plasma some other factor is concerned in preventing the increase of bicarbonate from corresponding with the reduction of chloride.

To illustrate further and explain the finding that bicarbonate increase is much less than would be the case were decrease in  $(\text{Cl}')$

the only factor determining ( $\text{HCO}_2'$ ), the measurements given in table 4 are presented graphically by means of the diagrams in figure 2. The left hand column represents the sodium concentration plus the usual average value for the sum of the other three plasma bases,

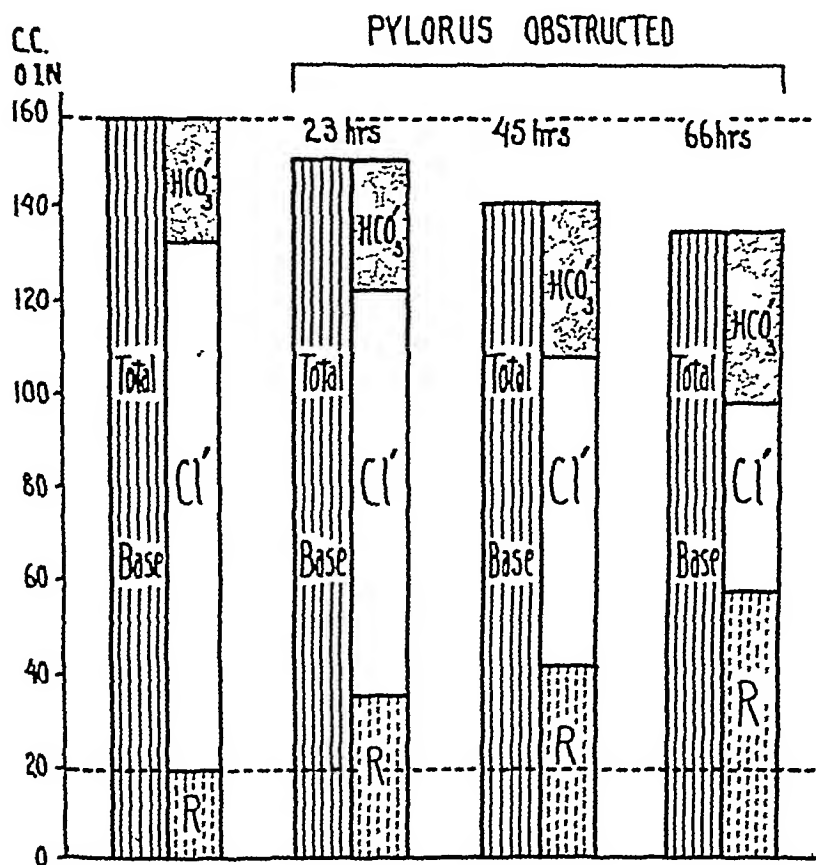


FIG 2 DIAGRAMS CONSTRUCTED FROM MEASUREMENTS GIVEN IN TABLE 4, ILLUSTRATING FACTORS DETERMINING THE BICARBONATE CONCENTRATION

potassium, calcium, and magnesium, taken as 12 cc 0.1 N per 100 cc of plasma. On the right side of the diagram is given first the ( $\text{HCO}_2'$ ) and then the ( $\text{Cl}'$ ) measurement. The remainder of the base column is balanced (see figure 1), by the relatively small concentrations of



$\text{HPO}_4''$ ,  $\text{SO}_4''$ , organic acids and the base equivalence of the plasma proteins. These factors, except in the case of  $\text{HPO}_4''$ , were not measured and their sum is designated  $R$  in the diagrams. It is at once apparent in figure 2 that there occurs a large and progressive increase of  $R$  and that this change exerts quite as large a limitation of the increase of  $(\text{HCO}_3')$  which tends to follow recession of  $(\text{Cl}')$  as does the loss of sodium. The broken lines across the diagrams indicate the extent to which reduction of base and increase of  $R$  respectively limit  $(\text{HCO}_3')$ . It will be clearly apparent from the diagrams that an enormous concentration of bicarbonate would result from the depletion of  $(\text{Cl}')$  were it not to a large extent prevented by these two changes in the acid-base balance of the plasma. From the data in table 4 it may be computed that, if  $(\text{Cl}') + (\text{HCO}_3')$  had remained in the plasma at the initial value, 139 cc 0.1 N per 100 cc, then  $(\text{HCO}_3')$  in sample no. 4 would have been 98 instead of 36 cc 0.1 N per 100 cc, or, in the more familiar form of statement, 203 instead of 81 volumes per cent of  $\text{CO}_2$ . Considering the possibility for bicarbonate increase represented by the very large reduction of chloride, the actual degree of alkalosis usually found following pyloric obstruction is, owing to these opposing factors, relatively small.

Explanation of this increase in  $R$  is not supplied by these data. The several measurements of phosphorus given in table 3 show that  $(\text{HPO}_4'')$ , one of the factors in  $R$ , was double its usual value in sample no. 4. But this factor is in the first instance so small (see figure 1) that this large relative increase explains to only a slight extent the total increase of  $R$ . Increase of the usually even smaller concentration of  $\text{SO}_4''$  to an extent appreciably altering  $R$  seems improbable. The usual value for the concentration of organic acid radicals en route for excretion is not accurately known. The single measurement of ketone acids obtained from sample no. 4 and given in table 4 indicates that there was no accumulation of these acids and indeed such accumulation would scarcely be possible following the administration of glucose solution. The most probable explanation of a considerable part of the increase of  $R$  is an increase of the base equivalence of the plasma proteins due both to the absolute increase in their concentration and to a shift presumably, in consequence of the bicarbonate

increase of the reaction of the plasma in the direction of alkalinity<sup>1</sup>. The extension of  $R$  is however so large that it does not seem probable that it can be entirely referred to an increase of base balanced by protein. There is thus suggested an increased concentration of organic acids.

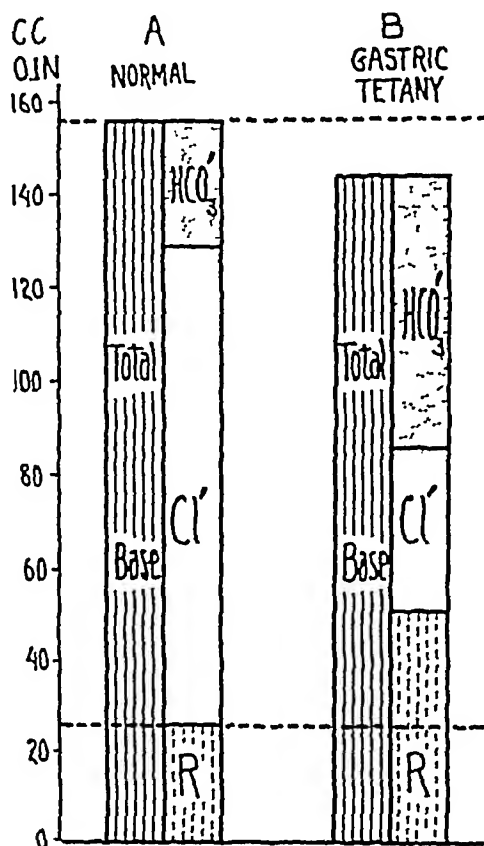


FIG 3 SHOWING RELATIONSHIP TO BICARBONATE CONCENTRATION OF LARGE CHANGES IN PLASMA FACTORS FOUND IN A CASE OF SEVERE GASTRIC TETANY

<sup>1</sup> The extent of the increase in plasma alkalinity following experimental pyloric obstruction in dogs is, according to the pH measurements published by Hastings, Murray and Murray (6), surprisingly small, even in the presence of a very large increase of bicarbonate.

To illustrate further the perhaps obvious point that the alkalosis which may follow obstruction of the pylorus is a consequence of the loss of  $\text{Cl}'$  from the plasma and to show again that extension of  $(\text{HCO}_3')$  from this cause is limited by loss of plasma base and by increase in some factor, or factors, contained in  $R$ , the diagrams in figure 3 were plotted (a), from average values obtaining in normal human plasma and (b), from values found in a sample of blood serum taken from a young man presenting severe symptoms of tetany following nearly complete pyloric obstruction caused by gastric ulcer<sup>4</sup> Here the increase in bicarbonate following the huge loss of chloride is much greater than was found in the experiments cited above, but is nevertheless considerably limited by loss of  $\text{Na}$  and increase in  $R$  Were all of the chloride loss replaced by bicarbonate the latter would be considerably greater (213 volumes per cent of  $\text{CO}_2$ ) than the very large value (130 volumes per cent of  $\text{CO}_2$ ) which was actually found

### *Experiment III*

Protocol Female dog Weight 10 20 kilos Abdomen opened under ether anesthesia and pylorus closed by tying with heavy silk Operation wound then closed and protected as in preceding experiments Beginning 24 hours after operation, four injections of sterile and warm 0.9 per cent sodium chloride solution, each of 400 cc, were given into the peritoneal cavity, the first three at 6-hour intervals and the last one after a 12-hour interval, i.e., 1600 cc of the sodium chloride solution were given during the first 48 hours following operation Beginning 96 hours (4 days) after the operation, three injections of sterile and warm 0.82 per cent ammonium chloride solution, each of 400 cc, were given into the peritoneal cavity at 6-hour intervals The dog was permitted to drink distilled water to such extent as desired Throughout the experiment it was kept in a metabolism cage and the large amounts of water containing gastric secretions which were vomited were collected over successive 24-hour periods The stomach was not emptied by tube at the end of each of these periods so that the collections per 24 hours were only approximate Urine was obtained by catheter during the first 5 days of the experiment at approximately 12-hour intervals It is believed that the collection of urine over this period was complete The dog during the 24-hour period of sodium chloride injections and for 2 days thereafter was bright and lively and apparently entirely comfortable, then gradually became dull and weak The injections of ammonium chloride solution produced no arrest of the increasing

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<sup>4</sup> The data used in constructing this diagram has been published by one of us (Ross) in a paper discussing the pathogenesis of gastric tetany (8)

apathy and feebleness. Twelve hours after the last injection and 6½ days after the operation the animal was killed with ether.

This experiment was undertaken with the purpose of testing the probability that the lowering of plasma base following pyloric obstruction is due to a loss of base as well as of chloride in the vomitus and also to demonstrate that introduction into the body, of NaCl solution, because it supplies both of the depleted ions will tend to restore the volume of body fluids, whereas administration of water containing a salt,  $\text{NH}_4\text{Cl}$ , which provides only chloride, will not check dehydration. The much greater structural significance of (Na) than of ( $\text{Cl}'$ ) has been discussed above. The urine specimens were collected for the purpose of obtaining data indicating the ability of the body to sepa-

TABLE 5  
*Measurements from vomitus, experiment III*

24 hour periods	Water drunk	Vomitus	pH	Cl	Na	K	Protein
	cc.	cc		cc 0.1 N	cc 0.1 N	cc 0.1 N	grams
1	2,100	1,970	4.5	504	289	0	0
2*	1,500	1,430	2.1	524	212	0	0
3	130	570	1.5	672	233	44	2.4
4	500	660	2.4	660	360	39	3.6
5	1,070	1,250	7.9	916	565	82	11.4
Totals	5,300	5,880		3,276	1,639	165	

\* 1600 cc. 0.9 per cent NaCl solution (= 2480 cc. 0.1 N) injected into the peritoneal cavity during this period.

rately and correctly retain (Na) and ( $\text{Cl}'$ ) in the body fluids from the administered NaCl.

In table 5 are given data obtained from five consecutive 24-hour collections of the vomitus which the animal provided after drinking the copious amounts of water. The collections during the remaining one and one-half days of the experiment were contaminated with urine and for this reason were not analyzed. That there occurs a very considerable amount of Na as well as of  $\text{Cl}'$  in the vomited stomach secretions may be seen at once in the table. In all except one of the specimens Na was found to an extent of somewhat more than half the

equivalence of the  $\text{Cl}'$  loss<sup>5</sup> During the fifth day of the experiment the dog began to grow dull and feeble after having been until then bright and lively The total of  $\text{Cl}'$  lost from the body in stomach secretions during the first four days of the experiment was, according to the measurements in table 5, 2300 cc 0.1 N which is approximately the amount (2480 cc 0.1 N) introduced into the body during the second day The estimations of fixed base excretion in the urine given in Table 7 make evident a large removal of Na by way of the kidney immediately following the injections of NaCl solution in addi-

TABLE 6  
*Measurements from blood serum samples, experiment III*

Time of taking sample	Bicarbo- nate	Chloride	Sodium	Calcium	Phos- phorus	Protein	Body weight
	vol. per cent $\text{CO}_2$	grams NaCl per liter	mg. per 100 cc	mg. per 100 cc	mg. per 100 cc	per cent	kilos
Before operation	47.0	5.99		11.0	5.8	7.0	10.20
1 day after operation	61.6	4.90		11.5	5.6	9.3	9.97
2 days after operation*	69.0	5.30	324	10.0	4.0	6.7	10.50
3 days after operation	70.0	5.67	310	9.8	5.8	6.4	10.18
4 days after operation	73.6	5.27	305		4.8	6.8	9.76
5 days after operation	81.0	4.29	280	10.3		7.9	9.12
6 days after operation †	44.3	4.70	294	10.4	11.0	8.9	8.94
6½ days after operation	56.0	4.33	274	11.9		9.6	8.74

\* Taken 6 hours after completion of administration of 1600 cc 0.9 NaCl solution

† Taken 6 hours after completion of administration of 1200 cc 0.82 per cent  $\text{NH}_4\text{Cl}$  solution

tion to the steady loss in the vomitus The onset of symptoms during the fifth day, roughly indicate that by then the benefit of the NaCl solution injected during the second day was dissipated by subsequent loss of the replaced elements The fifth day specimen was large, con-

<sup>5</sup> That is, the measurement of  $\text{HPO}_4''$  is stated in terms of the base equivalence indicated by the  $\text{BH}_2\text{PO}_4$  :  $\text{B}_2\text{HPO}_4$  ratio as determined by the pH of the specimen

The base equivalence of the organic acid excretion was estimated by subtracting a calculated value for free organic acids This was obtained by subtracting from a measurement of the titratable acidity of the specimen (to pH 7.4) the amount of  $\text{BH}_2\text{PO}_4$  in the specimen in excess of that which would obtain at pH 7.4, the data used here being the total  $\text{HPO}_4''$  excretion and the phosphate ratio corresponding to the pH of the specimen

The other acid radicals,  $\text{Cl}'$  and  $\text{SO}_4''$  carry, of course, their full equivalence of base into the urine

tained a much increased amount of Na and of Cl' and curiously, instead of being strongly acid as were the preceding, was markedly alkaline (pH 7.9). An appreciable amount of K was found in this specimen but the total of (Na) + (K) was far short of the (Cl') measurement. This specimen was moreover strikingly different in appearance from the preceding ones, being straw colored instead of presenting a faint whitish opalescence. It was also found to contain a large amount of protein (11.4 grams) and to this constituent may probably be referred the obliteration of HCl acidity.

The extent to which isotonic solutions of NaCl and of  $\text{NH}_4\text{Cl}$  respectively tend to support a normal composition of the blood plasma is indicated by the measurements of plasma values given in table 6. The attempts at measurement of (Na) in the first two specimens were unfortunately failures. It is probable, however, that some degree of depletion of (Na) had occurred during the 24 hours following operation. At any rate, following the injections of sodium chloride solution during the second day of the experiment, plasma (Na) was found to be 324 mg per 100 cc which is approximately its normal value and with which may be compared the measurement of 294 mg found at the same interval after operation in experiment II. The value of the injections of NaCl solution in restoring the water content of the body is well shown by the measurements of plasma protein and also by those of body weight. Benefit from the injections was strikingly apparent in the behavior of the animal. For two days following them, it was as bright and lively as a normal animal. Relief of thirst during these two days was indicated by the relatively small amount of water drunk.

During the fifth day there occurred a large reduction of plasma (Na) and (Cl') and a large increase in protein. As has been mentioned, the animal began to grow weak and dull. A marked increase in thirst was also evident. The injections of  $\text{NH}_4\text{Cl}$  solution were given during the next 24 hours. These entirely failed to arrest the increasing feebleness and apathy of the animal. As may be seen in table 6, the changes destroying the normal composition of the plasma continued. In spite of the 1200 cc of water given, dehydration of the plasma as indicated by the protein measurement, increased. This large amount of water was eliminated so rapidly that, during the 24 hours that it

was given, there occurred an actual loss of body weight. Following the first injection of  $\text{NH}_4\text{Cl}$  solution, urination became so frequent that separate collection of urine (by catheter) and vomitus was abandoned. In the last sample of serum obtained, owing to the increase in protein and  $(\text{Cl}')$  and the fall in  $(\text{Na})$ ,  $(\text{HCO}_3')$  was greatly reduced.

Measurements of calcium and phosphorus were made in most of the serum samples. Except for a relatively large increase in phosphorus in the sample obtained following administration of  $\text{NH}_4\text{Cl}$ , the values found for these factors were nearly stationary.

It is evident from these data that, although  $\text{NH}_4\text{Cl}$  may be expected to restore the loss of  $(\text{Cl}')$  the other disastrous alterations of

TABLE 7  
*Measurements of acid and base factors in urine, experiment III*

12 hour specimens	Volume	pH	Acid excretion in terms of base bound in urine						$\text{NH}_4$	Fixed base	Creatinine
			Organic acids	$\text{HPO}_4''$	$\text{SO}_4''$	$\text{Cl}'$	$\text{HCO}_3'$	Total			
			cc 0.1 N	cc 0.1 N	cc 0.1 N	cc 0.1 N	cc 0.1 N	cc 0.1 N	cc 0.1 N	cc 0.1 N	mg
No 1	58	6.6	68	120	94	44	3	329	78	251	190
No 2	46	5.7	65	74	29	4	1	173	82	91	170
No 3	75	6.3	69	99	56	10	2	236	116	120	180
No 4*	240	7.9	97	170	32	10	352	661	10	651	130
No 5	68	7.5	79	143	33	10	91	356	55	301	190
No 6	70	6.6	48	57	48	3	4	160	33	127	140
No 7	46	6.4	31	60	83	2	2	178	82	96	160

\* These two specimens were collected during period of administration of 1600 cc 0.9 per cent NaCl solution.

plasma structure will continue and may in fact be aggravated by the diuretic action of this salt. It must be admitted that the animal was in a condition of much more serious disrepair when the effect of  $\text{NH}_4\text{Cl}$  was tested than when NaCl was given. The character of the data obtained, however, clearly indicates the unsuitableness of  $\text{NH}_4\text{Cl}$  as a reparative agent. It may also be mentioned that the introduction of  $\text{NH}_4\text{Cl}$  solution into the peritoneal cavity was evidently irritating to a degree which should we believe, from the point of view of practical therapeutics, entirely forbid the giving of this salt by clysis.

There remains for brief discussion the data from consecutive 12-hour collections of urine covering the period of administration of NaCl

solution. These are presented in table 7 for the purpose of showing the removal of the excess of Na<sup>+</sup> over Cl<sup>-</sup> presenting for excretion in the urine owing to the larger deficit of (Cl<sup>-</sup>) than of (Na<sup>+</sup>) within the body. The usual situation, an excess of acid over fixed base claiming excretion which is met chiefly by a regulated production of ammonia is here reversed. The chief purpose of these data is to illustrate the fact that when fixed base claims excretion in the urine in excess of the sum of the acid radicals requiring removal the body possesses in carbonic acid a substance which is abundantly and adjustably available in defense of other acid components of the body fluids. Gamble (9) has shown that owing to a stationary concentration of H<sub>2</sub>CO<sub>3</sub> in the urine the amount of base which can enter as bicarbonate is a function of the pH at which urine is produced. At the usual pH of urine it cannot contain an appreciable amount of bicarbonate—a fact which excellently suits the usual need for an economy of base in the process of acid excretion. Adjustment of urine pH in the direction of alkalinity produces a rapid increase in the amount of bicarbonate entering the urine. These circumstances permit the use of carbonic acid as a means of conserving other acid components of the body fluids in a manner quite analogous to the defense of fixed base which the regulated availability of ammonia provides. The acid as well as the base side of the ionic structure of the body fluids is thus protected by the presence of a factor in the construction of urine which is widely adjustable.

The measurements of the acid factors in the urine are given (table 7) in terms of base bound at the reaction of the urine specimen. The amount of fixed base in the urine is taken as the difference between the sum of the acid factors and the ammonia measurement. The collections as regards 12-hour intervals were not sharp. Their character in this respect is indicated by the creatinine measurements. As may be seen in the table, Cl<sup>-</sup> is, except in the first specimen, permitted to enter the urine to only a very slight extent. Following the injections of NaCl solution there occurs a large but brief rise in the excretion of fixed base. This rise is seen to be chiefly balanced by an increase in HCO<sub>3</sub><sup>-</sup>. The data are presented graphically in figure 4, and by way of further illustration the acid-base composition of the specimen (no. 4) containing most of the excess of base removed is represented by the diagram A in figure 5. Diagram B in this figure shows the compo-



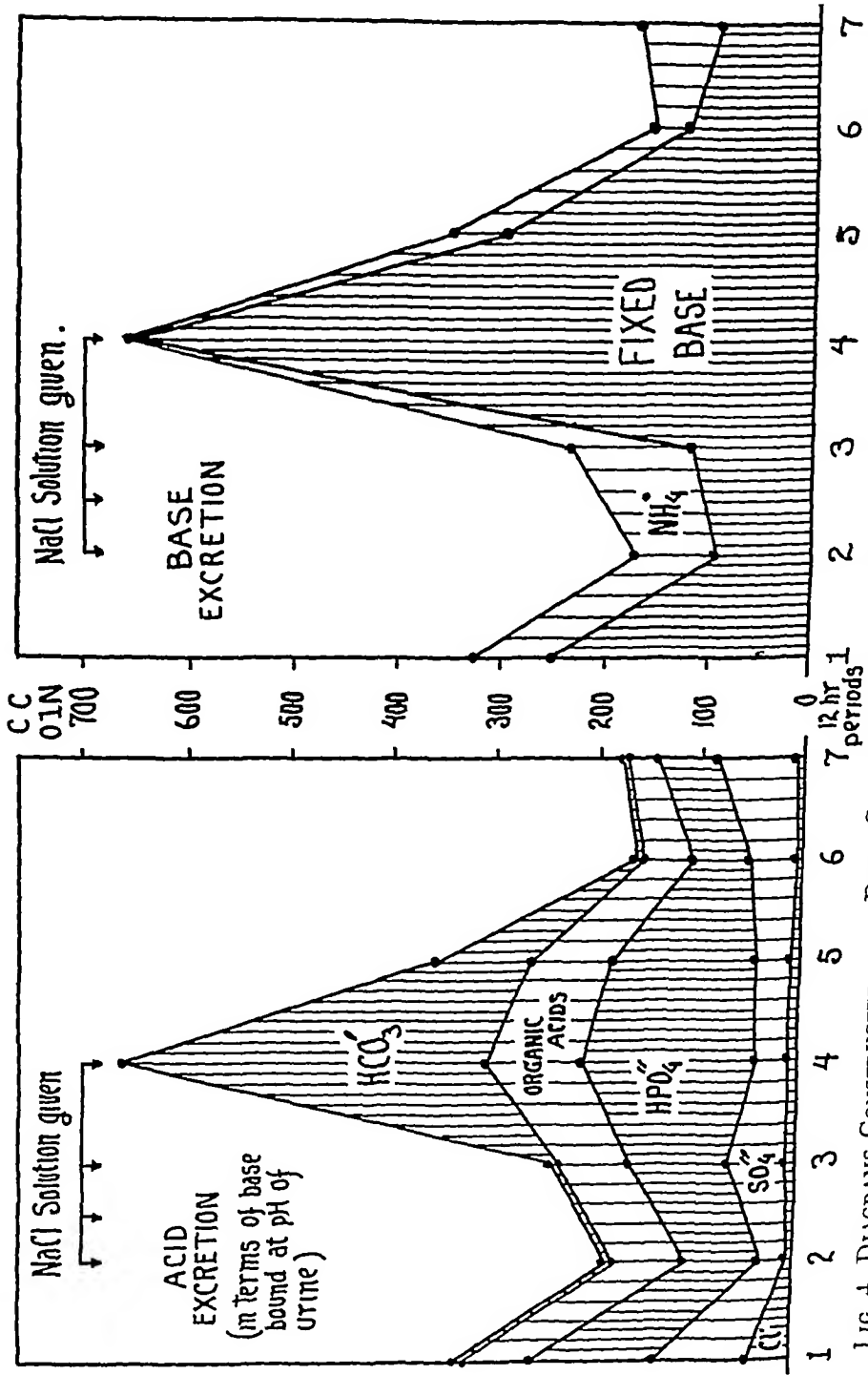


FIG. 4. DIAGRAMS CONSTRUCTED FROM DATA GIVEN IN TABLE 7 ILLUSTRATING USE OF HCO<sub>3</sub>' IN REMOVAL OF INCREASE OF N<sub>2</sub> PRESENTING FOR EXCRETION IN URINE FOLLOWING INJECTIONS OF NaCl SOLUTION

sition which might have been expected in this specimen had it been produced at the average usual reaction of urine pH 6.0. At this pH only a negligible amount of base can be carried into the urine as

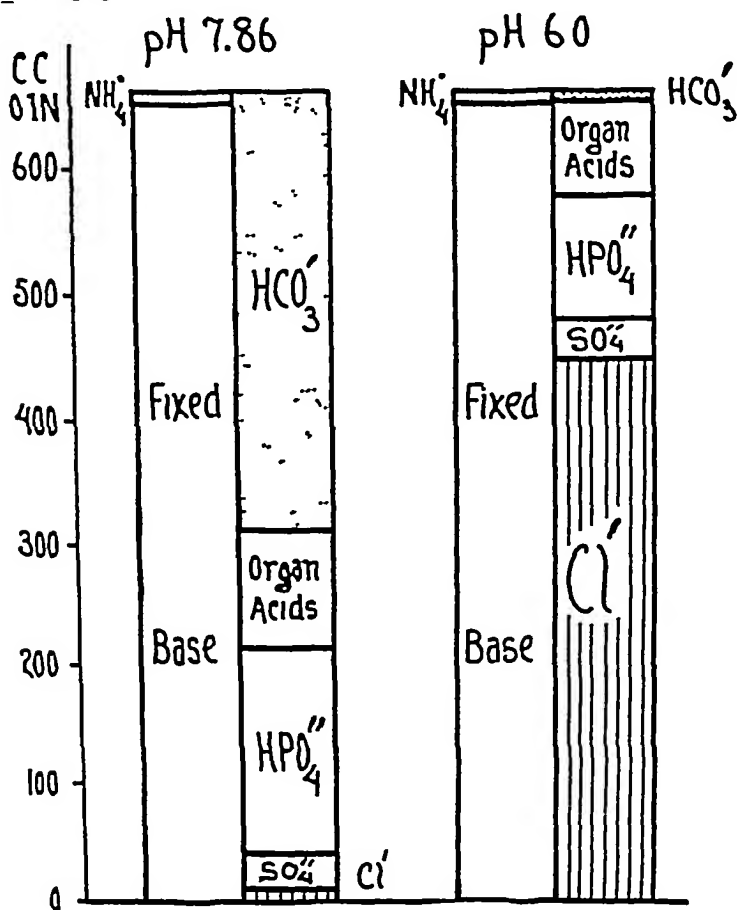


FIG 5 ILLUSTRATING THE EXTENT OF CONSERVATION OF  $\text{Cl}^-$  OBTAINED BY PRODUCTION OF SPECIMEN NO 4 (TABLE 7) AT pH 7.86 INSTEAD OF AT THE USUAL REACTION OF URINE, pH 6.0

$\text{BHCO}_3$  owing to the stationary value for the concentration of  $\text{H}_2\text{CO}_3$  in urine. Furthermore, the base equivalence of the organic acids and that of  $\text{HPO}_4^-$  is considerably less than at pH 7.86. The diagrams will serve to show clearly the magnitude of the changes in these

factors referable to pH and the consequent large saving of  $\text{Cl}'$  accomplished by the production of this specimen at pH 7.86 instead of at the usual reaction of urine

The secretion of an extremely alkaline urine following administration of this neutral salt clearly demonstrates the separate metabolism of its component ions

#### DISCUSSION

It will be noted that we have used in this study the point of view that the failure of physiological processes and death following experimental obstruction of the pylorus may be reasonably regarded as referable to the continued reduction of the volume of the body fluids in consequence of a progressive demolition of their sustaining structure caused by the loss of  $\text{Cl}'$  and more significantly of Na in vomited stomach secretions. The beneficial effect from introducing Na and  $\text{Cl}'$  into the body along with water is therefore regarded as being due simply to repair of this structure permitting recovery of a normal volume of body water. The data from these few experiments accords satisfactorily with this view.

In 1912, Hartwell and Hoguet (10) showed that following duodenal obstruction a dog could be kept alive for several weeks by replacing the loss of body water with physiological salt solution. They believed that the effect of the salt solution consisted simply in preventing dehydration. MacCallum and his co-workers demonstrated that following pyloric obstruction in dogs, the fall in plasma ( $\text{Cl}'$ ) and the increase in ( $\text{HCO}_3'$ ) with the accompanying symptoms of gastric tetany could be prevented by injections of isotonic NaCl solution.

Recently Haden and Orr (5, 11) have published extensive studies of plasma changes following experimental pyloric and upper intestinal obstruction. Their measurements show the large fall in chloride accompanied by more or less rise in bicarbonate and an increasing accumulation of the products of protein metabolism. They have tested the therapeutic efficacy of administration by clysis of a great number of salts:  $\text{KCl}$ ,  $\text{NH}_4\text{Cl}$ ,  $\text{CaCl}_2$ ,  $\text{MgCl}_2$ ,  $\text{KI}$ ,  $\text{NaBr}$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{MgSO}_4$ ,  $\text{NaH}_2\text{PO}_4$ , and  $\text{Na}_2\text{HPO}_4$ , and have found all of these to be of no benefit and, as is not surprising, often harmful (12). They have quite thoroughly demonstrated, however, that injection of NaCl solu-

tion tends to restore normal plasma values and greatly prolong the life of the animal (7). The loss of Na as well as of Cl', which we have shown, would apparently completely explain the unique action of NaCl as consisting simply in the fact that it is the only one of this long list of salts containing both of the ions specifically required for plasma repair. A much more elaborate explanation has been put forward by these authors. In interpreting the findings from their many experiments, they use the conception, derived from the work of Whipple, that the symptoms and death following obstruction are due to absorption of a toxic body. The accumulation of urea and of non-protein nitrogen in the plasma is not referred to renal disability following dehydration, but to rapid destruction of protoplasm caused by the poisonous agent. The lowering of (Cl') in the plasma they state can only in part be explained by loss in vomited secretions since it is found when vomiting is slight and also in rabbits, an animal which cannot vomit. They infer that Cl' leaves the plasma in quest of the toxic body. The benefit of the administered NaCl is therefore described as protective rather than reparative (13).

Obviously the few data given in this paper do not justify our contesting the views which Haden and Orr have developed from their extensive studies. However, they at least indicate, we believe, that the physical explanation (dehydration) of the events following pyloric or upper intestinal obstruction and of the beneficial effect of NaCl solution should be thoroughly tested as regards its sufficiency before accepting the additional and not easily credible hypothesis which these authors have devised.

#### SUMMARY

Following obstruction of the pylorus, there occurs in the vomited stomach secretions a loss of Na as well as of Cl' from the body. The sum of the concentrations of the acid radicals in the body fluids being determined, owing to adjustability of ( $\text{HCO}_3'$ ), by the fixed base concentration, a loss of Cl' does not deplete the total ionic concentration whereas loss of Na does and also removes an equivalence of  $\text{HCO}_3'$ . Reduction of the ionic content of the body fluids by withdrawal of Na is the significant factor in the rapid dehydration following pyloric obstruction. Dehydration cannot be repaired by the introduction

of water alone (glucose solution) or of water and the chloride ion ( $\text{NH}_4\text{Cl}$  solution) The efficacy of  $\text{NaCl}$  solution in sustaining the usual volume of body fluids is due to replacement of the loss of  $\text{Na}^6$

The increase of ( $\text{HCO}_3'$ ) in the plasma, which tends to occur following pyloric obstruction, is an automatic consequence of the depletion of ( $\text{Cl}'$ ) The degree of alkalosis is, however, usually greatly lessened by reduction of plasma base due to loss of  $\text{Na}$ , by extension of the base equivalence of plasma protein caused both by an increase in concentration of protein and of plasma alkalinity, and, possibly, by an increase in the concentration of organic acids

Following administration of  $\text{NaCl}$  solution, the surplus of  $\text{Na}$  over  $\text{Cl}'$  presenting for excretion, owing to the greater depletion of the latter, is conveyed into the urine as bicarbonate

#### CHEMICAL METHODS

**Blood serum** Using a Luer syringe, samples were taken from the jugular vein and then delivered through small bore glass tubing under oil into a centrifuge tube *Chlorides* were determined by a modification of the Van Slyke procedure essentially as described by Myers and Short (14) *Bicarbonate* was measured by the method of Van Slyke (15) and *ketone acids* by the method of Van Slyke and Fitz (16) The methods of Kramer and Tisdall were used in determining *sodium* and *calcium* (17, 18) *Phosphate* was measured by the method of Howland, Haessler, and Marriott (19), and *urea* by a micro modification of the urease method *Protein* was calculated from a determination of plasma refractivity according to Robertson (20)

**Vomitus** *Sodium* and *potassium* were determined in ashed samples by the methods of Tisdall and Kramer (21) and chloride as in blood serum *Protein* was calculated from a Kjeldahl measurement of nitrogen and *pH* was determined colorimetrically

**Urine** The specimens were obtained by catheter and were collected under oil *Bicarbonate* was calculated from measurements of total  $\text{CO}_2$  and of *pH* according to Gamble (8) *Phosphates* (inorganic) were determined by the uranium acetate method *Sulphates* (inorganic) were weighed as barium sulphate according to the method of Folin (22) *Chlorides* were determined as in blood serum The organic acid excretion was measured by the method of Van Slyke and Palmer (23) *pH* was determined colorimetrically under oil, using the comparator method of compensating for the color of the specimen The measurements were made at room temperature

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<sup>6</sup> Although it is here argued that loss of  $\text{Cl}'$  is not significant as regards the causation of dehydration, its replacement along with  $\text{Na}$  is obviously necessary in order to prevent alkalosis

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# THE RESISTANCE OF IMMATURE ERYTHROCYTES TO HEAT\*

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## INTRODUCTION

Knowledge concerning the differences between mature cells and those in the process of development adds to the understanding of the vital problem of growth. The red blood corpuscles offer excellent material for the study of adult and immature forms, because of the recognition of four successive stages in their development. These include the nucleated red cells, the reticulated forms, the granule red cells (cells described by Isaacs, with single, refractile, non-staining granules) and finally the mature erythrocytes. Heat as a "cytotoxic" agent does not appear to have been utilized, heretofore, for the purpose of studying different kinds of erythrocytes.

The destructive and alterative action of heat on erythrocytes was described some sixty years ago by Klebs, Rollett, Beale and Schultze. Since then there appear but few papers on this subject, among them are those quoted by Krehl and Marchand. No quantitative data, however, have been found, nor any observations on the behavior to heat of erythrocytes in different diseases. The present study was undertaken to discover whether the red cells in various diseases reacted differently towards heat, and to learn if there was any quantitative relationship between the ages of red cells and their resistance to heat.

\* This paper is No. 40 of a series of studies in metabolism from the Harvard Medical School and allied hospitals. The expenses of this investigation have been defrayed in part by a grant from the Proctor Fund of the Harvard Medical School, for the Study of Chronic Disease, and in part by the Edward Hickling Bradford Fellowship.

† Bradford Fellowship.



## METHODS AND MATERIAL

The following procedures were utilized in studying the effect of heat on the red blood corpuscles of patients and normal persons

Blood from an arm vein was mixed with sufficient crystalline sodium citrate to make a 0.2 per cent solution. Blood films, supravitaly stained with brilliant cresyl blue and counterstained with Wright's stain were made on cover glasses. Such preparations were used to study the condition of the blood before and after it had been subjected to heat. One cubic centimeter of blood was heated in small test tubes, suspended in a water bath at a temperature ranging from 55° to 58°C. Whenever a pathological blood was tested, blood from a healthy person, as a control, was treated at the same time in an identical manner. Every care was taken to prevent further alteration of the blood by such physical means as mixing, shaking or foam formation.

Capillary glass tubes, washed free of alkali were used in other experiments and blood was taken directly into them from a skin puncture, and, as it ran from the wound, was mixed with a few crystals of sodium citrate. Then the tubes were sealed and heated in a water bath. There was no appreciable difference in the results obtained from these two methods.

In addition to blood from five healthy individuals, used as controls with each heating, blood from 31 patients (28 with anemia and 3 of erythremia with polycythemia) were studied. All 31 of the patients showed a percentage of immature red cells above normal. The 28 patients with anemia included 8 cases of chronic myelogenous and 3 of chronic lymphatic leukemia, 6 of Hodgkin's disease, 3 of pernicious anemia in relapse, 2 of chronic thrombopenic purpura, 4 of familial (one splenectomized) and 1 of acquired, chronic hemolytic jaundice and 1 of hemolytic anemia of pregnancy.

## EFFECT OF HEATING NORMAL WHOLE BLOOD

The red cells in normal blood subjected to heat up to 50°C for 30 minutes show little or no visible change. At 65°C and higher they are destroyed almost completely. Normal blood when heated to 55°C for 30 minutes, shows a marked alteration in the shape and

appearance of the red cells (fig 1) Seventy to 80 per cent of the cells are broken up, or appear as mere shadows, or present unusual

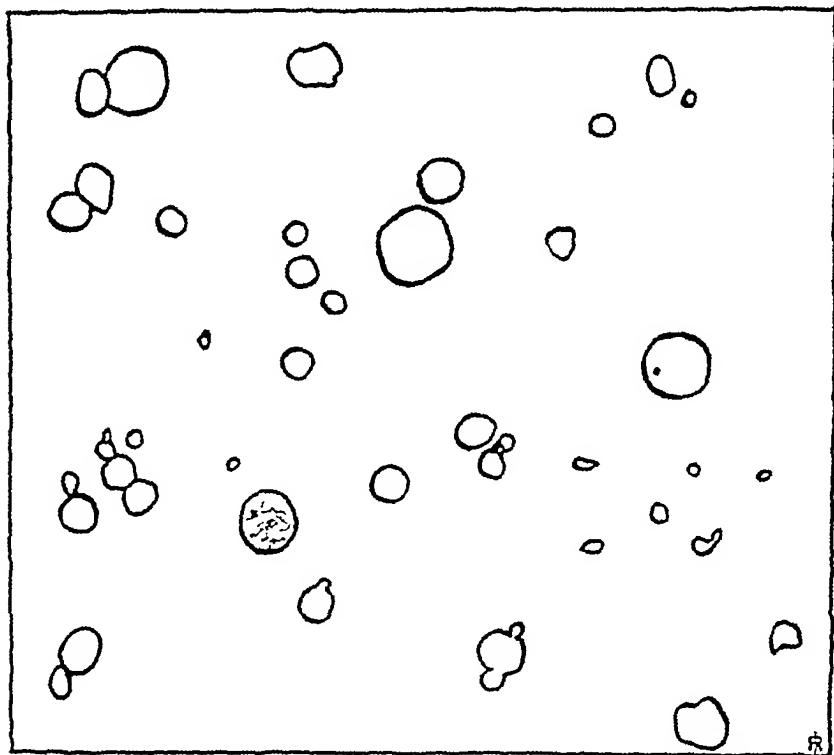


FIG 1 REPRESENTATIVE FIELD OF A COVERGLASS FILM OF NORMAL BLOOD WHICH PREVIOUSLY HAD BEEN HEATED TO 55°C FOR 30 MINUTES

A granule red cell and a reticulated red cell (basophilic) are shown. Fragmentation is marked, and but few of the cells are intact. The stroma of the hemolyzed red corpuscles is not shown in the drawing. It is visible in the stained preparations and the "shadows" fill the spaces between the intact cells. This figure and figure 2 represent microscope fields in which the total number of intact cells plus 'shadows' is approximately equal.

Camera lucida drawing (table level) Leitz ocular 8, oil immersion objective 2 mm

and bizarre forms. Spherical bodies resembling microcytes are a feature, and smaller irregular fragments and filaments of cells are numerous. Many larger cells, less affected, are deformed and elon-

gated A few cells are apparently little altered, but practically all lose their normal staining characteristics The cells stain uniformly, and homogenously, and perhaps are somewhat more basophilic than normal The reticulum of the reticulocytes, as Key observed, does not stain so well as in unheated specimens The microcyte formation is present before films are made and is not produced by trauma

The cells which broke up in greatest numbers were the mature cells, lacking the structures (reticulum, granules) that characterize young cells while the immature cells remained intact Table 1 shows the effect of heating a sample of normal blood Among the intact cells the percentage of immature ones is strikingly increased The absolute number of young cells before and after heating was approximately the same (within experimental error) so that it is not likely that heating produced forms resembling the different types of immature

TABLE 1

*Effects of heating specimen of normal blood to 55°C for thirty minutes*

(Numbers represent the percentage of intact red cells)

	Before heating	After heating
Reticulated and basophilic red cells	1 4	15 1
Granule cells	1 8	26 3
Total recognizable young red cells	3 2	41 4

cells Similar results were obtained repeatedly, though the actual number of intact cells varied In blood remaining overnight at 4°C or at 37°C all red cells, especially reticulocytes, became more resistant to heat than fresh corpuscles These observations lead to the conclusion that *normal immature red blood corpuscles are more resistant to heat than adult corpuscles*

#### EFFECT OF HEATING PATHOLOGICAL BLOODS

The observation that immature red cells, including blasts, are more resistant to heat than adult red cells was brought out much more strikingly in studying the 31 pathological bloods containing an increased (often high) percentage of reticulocytes Scrutiny of many preparations showed that reticulated microcytes, macrocytes and normocytes resisted the action of the heat in the same manner

Here appeared to break up reticulated megalocytes in two cases of pernicious anemia. Actual measurements of cells indicated that red cells shrink during heating, but this contraction alone does not seem to account for the disappearance of the extremely large corpuscles. Thus the actual size of the cell, unless a megalocyte, plays no rôle in its resistance to heat. *The only differences produced by heating the blood from the patients with the various diseases were quantitative (proportional to the degree of immaturity) and no specific qualitative differential criteria were noted.* In some bloods containing a high percentage of recognizable immature red cells, there remained, after heating, a relatively large number, compared to normal, of cells not containing nuclei, reticulum or granules. This was particularly true of the blood from cases of chronic hemolytic jaundice.

The blood from four untreated cases of chronic hemolytic jaundice showed a greatly increased fragility to hypotonic salt solution of the red blood corpuscles and a distinct increase of the reticulocytes, both of which features are characteristic of this condition. Owing to the decreased osmotic resistance of the red cells one might suppose that their destruction by heat would occur more readily than normal. Such is not, however, the case. In chronic hemolytic jaundice the response of the red cells to heat confirms the view that the immature cells are more resistant than the mature cells to this physical agent. The data recorded in table 2, illustrate the effect of heat on the red cells of this condition as contrasted with the normal. As the table shows, the bulk of the red cells resist destruction. The number of intact cells per microscope field (oil immersion) in the control (A) blood film after heating was, on the average, for 50 fields, 9.9, while in comparable films of the hemolytic jaundice blood (A), the average number of intact cells was 238 (fig. 2). In spite of the differences in figures for such data as shown in table 2 obtained by carefully observing and counting both intact and broken cells for controls and cases, the resistance to heat of the bulk of the red cells in chronic hemolytic jaundice is distinct enough perhaps to aid in diagnosis.

Red cells from the blood of normal persons and of patients with chronic hemolytic jaundice were mixed with 0.7 and 0.85 per cent sodium chloride solution and studied before and after heating. Here another factor than heat influences the results. The red cells of a

particular patient's blood were not hemolyzed before heating by 0.7 per cent sodium chloride solution, but were hemolyzed in small numbers by 0.6 per cent salt solution. Normal cells were similarly affected by 0.42 per cent salt solution. After heating, this patient's cells mixed with 0.7 per cent sodium chloride solution practically all

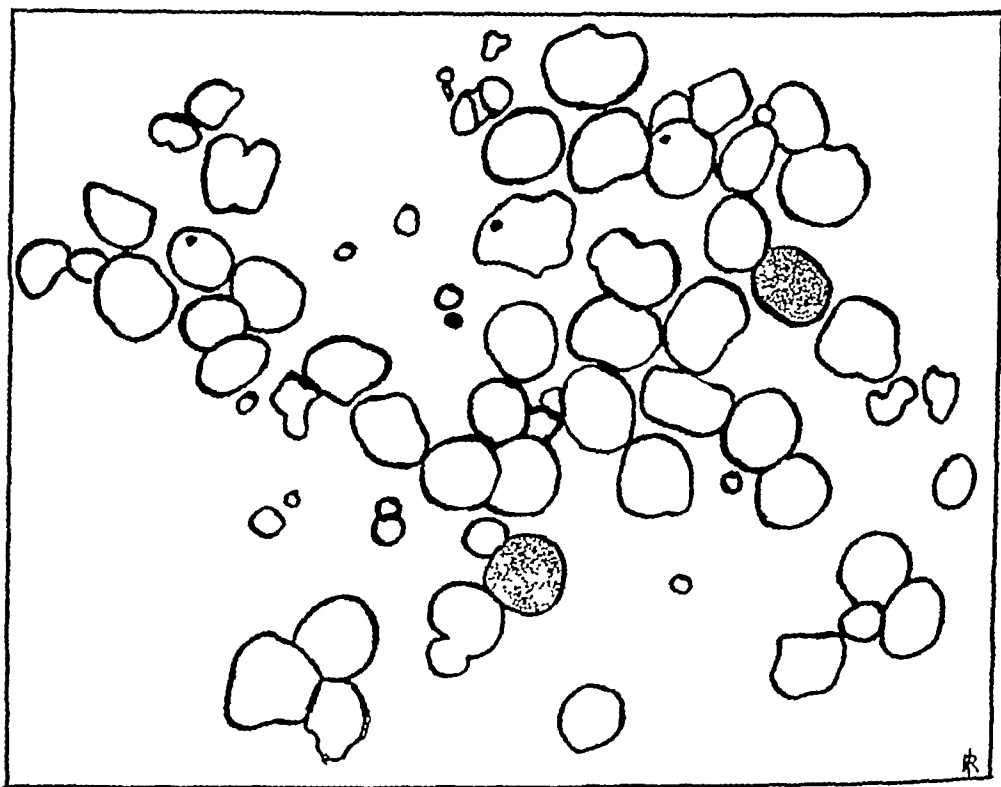


FIG 2 REPRESENTATIVE FIELD OF A COVERGLASS FILM OF BLOOD AFTER HEATING TO 55°C FOR 30 MINUTES, FROM A PATIENT WITH CHRONIC HEMOLYTIC JAUNDICE

Three granule red cells and two reticulated red cells (basophilic) are shown. Compared to normal (fig. 1) the number of intact cells is greatly increased and fewer fragments are evident. Drawn like figure 1.

were hemolyzed, when 0.85 per cent was used fewer cells were hemolyzed. This indicates that cells already fragile to hypotonic salt solution tend to be destroyed by the combined effect of the solution and heat. The combined effect of 0.7 per cent salt solution and heat on normal cells resulted in the destruction of more cells, than when heat was allowed to act upon them in their own plasma or in 0.85

per cent salt solution, but the effect upon normal cells was not nearly so marked as the effect upon the patient's red cells treated in the same manner

It is well recognized that with the increased red cell destruction, and constant renewal of these corpuscles in the circulation in chronic hemolytic jaundice, the blood is filled with varying, but increased numbers of recognizable young cells. The life of the mature red cell

TABLE 2

*Comparison of specimens of normal blood with blood from two cases of chronic hemolytic jaundice, showing the effect of heat*

	Normal (control)	Chronic hemolytic jaundice
Red blood cell count (millions per cubic millimeter)	(A) 4.8 (B) 5.1	(A) 2.50 (B) 3.98
"Fragility" to hypotonic salt solution		
Hemolysis began (per cent)	(A) 0.40 (B) 0.42	(A) 0.54 (B) 0.60
Hemolysis complete (per cent)	(A) 0.30 (B) 0.34	(A) 0.36 (B) 0.36
Per cent of red cells showing basophilia, reticulation or granules <i>before heating</i> in plasma	(A) 3.0 (B) 3.3	(A) 21.0 (B) 17.0
Per cent of intact red cells showing basophilia, reticulation or granules <i>after heating</i> in plasma	(A) 38.5 (B) 60.0	(A) 39.1 (B) 56.8
Per cent of intact red cells (after heating) of original total (estimated)	(A) 10.0 (B) 10.0	(A) 60.0 (B) 50.0
Average number of intact red cells per oil immersion field <i>after heating</i> in plasma.	(A) 9.9 (B) 14.6	(A) 238.0 (B) 60.0

in this disease is probably shorter than normal, because of the increased destruction of corpuscles. There thus should appear in the blood in this condition, a relatively larger number of *young mature* corpuscles, and comparatively few old ones, the latter presumably being the cells first removed by hemolysis. As young red cells are more resistant to heat than mature cells, those adult cells which resemble the immature ones in their heat resisting ability, may well

be the youngest of the mature cells. These are the cells which are so much more plentiful in the blood of chronic hemolytic jaundice than normal and which may occur in the blood of other pathologic conditions though never in such profusion. Differences in the character and rate of blood destruction and maturation and delivery of red cells can account for varying numbers of heat resistant adult erythrocytes in the circulation.

### SUMMARY

1 The red blood corpuscles of normal human blood, when heated to 55°C for one-half hour, undergo a profound and characteristic modification, with the production of fragmented forms, "shadows," microcytes, poikilocytes and a uniform distribution of the hemoglobin throughout the cell.

2 Immature erythrocytes—reticulocytes, polychromatophilic cells and granule red cells—of both normal and pathological blood are broken up less readily when heated to 55°C than mature erythrocytes. The reticulated megalocytes are apparently an exception.

3 The difference between the effect of heat on the red cells of normal and pathological blood is not qualitative, it is quantitative, proportional to the number of immature cells.

4 There are two kinds of red cells which show no histological evidences of immaturity, and are classed as mature corpuscles. One kind resists the action of heating to 55°C while the other is broken up and altered. The former represents a majority of the red blood corpuscles in chronic hemolytic jaundice. They are probably the younger of the mature cells.

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# THE PLASMA PROTEINS IN RELATION TO BLOOD HYDRATION

## I IN NORMAL INDIVIDUALS AND IN MISCELLANEOUS CONDITIONS

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### INTRODUCTION

During the last two years an extensive investigation has been made of the water and electrolyte changes occurring in the blood in nephritis and diabetes and in other diseases which presented analogous phenomena. As part of this study the oxygen-capacity, cell volume and plasma proteins have been determined simultaneously in an attempt to gain some insight into the causes of the variations in the protein content of the serum and their relation to the hydration of the blood and the tissues.

Most of the determinations of blood proteins reported in the literature have been carried out on serum. In the present studies plasma has been employed almost exclusively. At first sight one would expect the values obtained from plasma to be somewhat higher than those from serum, because fibrinogen has been removed from the latter. That this is not actually the case has been shown by Gettler and Baker (5). They found that the total nitrogen of serum was somewhat greater than that of plasma. The apparent paradox may be explained by the fact, demonstrated by Gram and Norgaard (6) and others, that the addition of anticoagulant amounts of oxalate to blood causes a shrinking of the cells.

Eisenman and Peters (4) have shown that this effect is demonstrable and amounts to a change of about 2 volumes per cent of cell volume, even if only 0.2 per cent of neutral potassium oxalate is used. In an attempt to verify the actual value of the change they compared cell

volume and plasma protein values The results of these experiments appear in table 1

### METHODS AND RESULTS

Cell volumes were determined in duplicate with a Daland hematocrit of the type manufactured by the International Instrument Company for their centrifuges With this instrument duplicates usually check within one volume per cent In order that the effect of fibrinogen might be eliminated and that only the effect

TABLE 1

Case number	Oxygen capacity	Cell volume	Serum proteins	Relative change of serum volume		Treatment of serum
				From oxygen capacity and cell volume	From serum proteins	
	<i>vols per cent</i>	<i>vols per cent</i>	<i>per cent</i>			
29123	20 27	48 41	7 24*	1 00	1 00	Without oxalate
	20 47	43 58	6 92*	1 08	1 05	With 0.2 per cent potassium oxalate
29397	19 76	45 55	6 67	1 00	1 00	Without oxalate
	19 76	43 55	6 28*	1 04	1 06	With 0.2 per cent potassium oxalate
18433	10 05	23 95	6 83	1 00	1 00	Without oxalate
	10 10	20 35	6 44*	1 05	1 06	With 0.2 per cent potassium oxalate

\* Non-protein nitrogen was not determined on these samples, but the values for total nitrogen, in terms of per cent protein, were reduced by 0.19, corresponding to a blood non-protein nitrogen of 30 mg per 100 cc, or were assumed to be identical with those of the specimens without oxalate

of oxalate might be active a specimen of blood was first defibrinated To one portion of the blood neutral potassium oxalate was added Both samples were brought into equilibrium with 40 mm of CO<sub>2</sub> in air at 38°C A portion of each was then used for the determination of oxygen capacity and cell volume, from another portion the plasma was removed for analysis All procedures were carried out by the technique previously described (9), to prevent exposure to air In this way variations in cell volume which might have resulted from differences of CO<sub>2</sub> tension were avoided The total nitrogen of each sample of plasma was determined in duplicate by a macro-Kjeldahl method, using  $\frac{1}{2}$  cc of plasma In some instances no correction was made for the non-protein nitrogen of the plasma.

Instead a blood non protein nitrogen value of 30 mg per 100 cc was assumed and the total nitrogen of the serum was reduced accordingly. This course was pursued throughout this work in the interests of economy. Whenever there was any reason to suspect a change in the non-protein nitrogen the latter was separately determined. The error introduced by the omission of this procedure probably never exceeds 0.1 per cent.

It is evident that in every case the addition of ovalate to the blood results in a contraction of the cells and a reduction of the serum protein concentration. The change in volume of the serum calculated from the serum protein values is of the same order of magnitude as that calculated from cell volumes and hemoglobin. This is quite in keeping with the theory that the cell membrane is impervious to proteins.

TABLE 2

Subject	CO <sub>2</sub> tension	Cell volume	Plasma proteins	Relative plasma volume from cell volume	Relative plasma volume from plasma proteins
	mm	vols per cent	per cent		
J P 5a	30	41.4	6.59*	1.00	1.00
	60	41.5	6.65*	0.993	0.991
J P 5b	30	52.0	9.16*	1.00	1.00
	60	52.2	9.17*	0.996	0.999

\* Non protein nitrogen was not determined on these samples, but the values for total nitrogen, in terms of per cent protein, were reduced by 0.19, corresponding to a blood non-protein nitrogen of 30 mg per 100 cc.

The average diminution of the proteins, 0.37 per cent, is equal to or possibly a little greater than the average normal concentration of fibrinogen in the plasma.

As changes in CO<sub>2</sub>-tension are known to cause alterations in the volume of the cells one would expect corresponding alterations in the plasma proteins. The difference in both cell volume and plasma proteins produced by varying the CO<sub>2</sub>-tension between 30 and 60 mm is shown in table 2. At least in this experiment it is negligible. If we assume that there is no interchange of protein across the cell membrane in vitro the variations of protein produced can be calculated from the change in cell volume alone, for which considerable data are available. In 34 experiments the average cell volume at 30 mm of CO<sub>2</sub> was 40.5 volumes per cent, at 60 mm it was 41.2 volumes per

cent The plasma volume therefore varied from 59.5 volumes per cent at 30 mm to 58.8 volumes per cent at 60 mm. This would produce a difference of about 1 per cent in the concentration of the proteins, which would be greater at a higher tension. Variations in oxygen will, also, of course, affect the cell volume and therefore the concentration of plasma proteins. The quantitative effect of oxygenation and reduction has not been investigated by us, but the work of Warburg (14) and of Van Slyke, Wu and McLean (13) indicate that it is relatively small. In general it may be said that the changes in  $\text{CO}_2$  and  $\text{O}_2$  tension usually encountered in the blood have a demonstrable but inconsiderable effect on the cell volume and on the plasma proteins.

TABLE 3

Subject	Oxygen capacity	Cell volume	Plasma proteins	Relative plasma volume calculated		Remarks
				From oxygen capacity and cell volume	From plasma proteins	
	<i>vols per cent</i>	<i>vols per cent</i>	<i>per cent</i>			
J P 1	18.61	42.7	6.90*	1.00	1.00	Without stasis
	22.45	51.2	9.32*	0.71	0.74	With stasis
J P 2	18.42	41.5	6.62*	1.00	1.00	Without stasis
	22.72	52.1	9.17*	0.66	0.72	With stasis

\* Non-protein nitrogen was not determined on these samples, but the values for total nitrogen, in terms of per cent protein, were reduced by 0.19, corresponding to a blood non-protein nitrogen of 30 mg per 100 cc.

In 1915-16 Rowe (10) demonstrated the fact that the production of venous stasis in a limb increased the plasma proteins in the venous blood of the part. In his experiments the serum albumin increased more than the globulin. This led him to believe that the change could not be due only to a transfer of water from the blood to the tissues. That such a transfer does occur has been suggested by Dautrebande, Davies and Meakins (3) and by one of us (8).

In the two experiments presented below (table 3) venous stasis was produced in the arm of a normal individual by means of a tourniquet which was applied with sufficient pressure to obstruct the venous return without completely obliterating

the arterial pulse. Blood was withdrawn from the arm vein immediately before the tourniquet was applied and again after the tourniquet had been in place for about five minutes.

In these and other similar experiments a considerable increase in hemoglobin, cell volume and plasma proteins occurred. If it is assumed that these changes were due only to inspissation of the blood, the amount of water lost to the tissues may be calculated. From the hemoglobin and hematocrit values it appears that the blood has lost considerable water predominantly at the expense of the plasma. If the change of plasma volume be calculated from the differences in plasma protein figures of the same order of magnitude are obtained. It seems hardly reasonable to doubt that the chief mechanism responsible for these alterations in proteins and blood cells is loss of fluid to the tissues and that this fluid carries with it little or none of the proteins. To be sure the plasma volume changes calculated from hemoglobin and cell volume do not in either case agree exactly with those derived from the protein values<sup>1</sup>. In these two experiments the latter method indicates less change, which suggests that a part of the protein has escaped from the vessels. In other similar experiments discrepancies of the opposite sign have been found. It is, of course, possible that an exchange of proteins may occur in either direction, but it is just as reasonable to suppose that the stasis has tended to filter out a certain proportion of the blood cells in the capillaries.

The agreement between the hemoglobin-hematocrit and the plasma protein calculations is so close that one is almost forced to disregard the slight variations in *albumin-globulin* ratio found by Rowe (10) and to believe that the increases of plasma protein resulting from venous stasis are due to a concentration of the blood which affects predominantly the plasma and that even under the drastic conditions of these experiments the vessels remain practically impermeable to the proteins.

Rowe (12) found that the serum protein concentration was increased by exercise and Barr and Himwich (2) have noted a similar rise of hemoglobin. In some experiments of which one is submitted in table

<sup>1</sup> The failure to correct for non-protein nitrogen is recognized as a source of some error, but is probably negligible.

4 the changes observed have been neither as considerable nor as constant as those reported by Rowe and by Barr and Himwich. Their experiments differed from ours in one respect especially. They withdrew the blood immediately after the cessation of general exercise. In the experiment here presented blood was taken from the veins of the exercised part during the course of the exercise.

Both arms and forearms were thoroughly warmed, before the experiment, by immersion in hot water. The right hand was then exercised by the alternate extension and flexion of the wrist and hand with a weight of about ten pounds suspended from the tips of the fingers. When the exercise had been continued to the point of painful exhaustion blood was simultaneously withdrawn from the veins of both arms without stasis.

Table 5 shows the results of over-ventilation carried to the point where tetany developed.

Both hands and forearms of the subject were warmed thoroughly by immersion in hot water. Venous blood was then withdrawn from the arm vein. Immediately after the blood had been obtained the subject began breathing as hard as possible. Definite carpo-pedal spasm and other evidences of tetany developed in about five minutes. When these symptoms had become quite marked a second sample of blood was taken from the same arm vein. Over-ventilation was continued until the blood had been removed.

Although changes occur in hemoglobin, cell volume and plasma proteins there is nothing characteristic in them. It would, in all probability, be possible to multiply examples of conditions associated with alterations in blood water *ad infinitum*.

Dautrebande, Davies and Meakins (3) have demonstrated the fact that cold, stasis and certain diseased conditions, notably cardiac decompensation, cause the blood to give up an excessive amount of fluid to the tissues. So rapid are these changes that they may result in the production of appreciable differences in hemoglobin, cell volume and plasma proteins between arterial and venous blood. One of us (8) has already presented data confirming this work and showing that under other circumstances the blood may gain water from the tissues.

Whether these changes in plasma protein are expressions of alteration in blood hydration or not, their bearing on the determination of plasma protein values is the same. If plasma proteins are to be

TABLE 4

Subject	Oxygen capacity	Cell volume	Plasma proteins	Relative plasma volume calculated		Remarks
				From oxygen capacity and cell volume	From plasma proteins	
	<i>rels per cent</i>	<i>rels per cent</i>	<i>per cent</i>			
J P 1	18 30	38 1	6 60*	1 00	1 00	Venous blood from unexercised arm
	18 74	41 4	6 67*	0 93	0 99	Venous blood from exercised arm
J P 2	18 21	42 6		1 00		Venous blood from unexercised arm
	18 73	43 9		0 95		Venous blood from exercised arm

\* Non-protein nitrogen was not determined on these samples, but the values for total nitrogen, in terms of per cent protein, were reduced by 0.19, corresponding to a blood non-protein nitrogen of 30 mg per 100 cc

TABLE 5

Subject	Oxygen capacity	Cell volume	Plasma proteins	Relative plasma volume calculated		Remarks
				From oxygen capacity and cell volume	From plasma proteins	
	<i>rels per cent</i>	<i>rels per cent</i>	<i>per cent</i>			
J P 1	19 11	42 1	6 55*	1 00	1 00	Venous blood before over ventilation
	18 78	41 2	6 67*	1 03	1 00	Venous blood during over ventilation
J P 2	18 86	43 9	6 76*	1 00	1 00	Venous blood before over ventilation
	19 87	43 9	7 27*	0 95	0 93	Venous blood during over ventilation

\* Non-protein nitrogen was not determined on these samples, but the values for total nitrogen in terms of per cent protein, were reduced by 0.19, corresponding to a blood non-protein nitrogen of 30 mg per 100 cc



interpreted with any degree of accuracy determinations must be made under standard conditions with precautions to avoid stasis, cold, exercise and any other unusual circumstances. Arterial plasma is preferable to venous plasma in most cases.

The values for the plasma proteins of the venous blood of five normal individuals, members of the hospital and laboratory staff, in table 6, agree with those published by other observers and are quite variable. Unfortunately the blood from the first four subjects and the first four specimens from the last subject were taken with no special

TABLE 6

Subject	Date	Plasma protein
		<i>per cent</i>
H A B		7.25*
		7.98*
K B		7.92*
H J S		7.61*
M S		6.52*
J P P	November 28, 1922	6.70*
	January 22, 1923	6.72*
	March 20, 1923	6.94*
	March 11, 1924	6.71*
	March 18, 1924	6.43*
	March 27, 1924	6.42*
	April 3, 1924	6.41*
	April 10, 1924	6.46*
	April 16, 1924	6.57*

\* Non-protein nitrogen was not determined on these samples, but the values for total nitrogen, in terms of per cent protein, were reduced by 0.19, corresponding to a blood non-protein nitrogen of 30 mg per 100 cc.

precautions and much of the variation may be due to this fact. The five last specimens from J P were all taken without stasis at approximately the same time of day, after a preliminary rest period and after the arm and hand had been warmed by immersion in hot water. The constancy of the proteins in these experiments is quite striking in contrast to the previous ones. In some of these experiments the plasma proteins were determined after the blood had been brought into equilibrium at 38°C with 40 mm of CO<sub>2</sub> in air. In the others the blood was centrifuged and the plasma removed with precautions to prevent all contact with the air. There is no systematic difference

between the two sets of figures, confirming the previous statement that the changes of gas tension in the blood encountered under ordinary circumstances have no significant effect on the plasma proteins

Such normal figures are hardly comparable to those obtained from patients in the ward, restricted in their activities and diet and presenting too often a variety of functional disturbances that may affect the water content and the plasma proteins of the blood. Before attacking the problems of diabetes, nephritis and edema, therefore, it seemed advisable to study patients with miscellaneous pathological conditions. Some of these were selected as control material because there seemed no reason to expect any disturbance of the plasma proteins, others because it seemed quite possible in the light of previous reports or certain clinical phenomena that the proteins might be abnormal.

The technique followed was similar to that described above. In some cases venous blood was used, in others arterial. Sometimes the blood was brought into equilibrium with 40 mm of  $\text{CO}_2$  in air at  $38^\circ\text{C}$ , sometimes it was withdrawn and analyzed with precautions against contact with air. The nature of the blood (whether venous or arterial) and its treatment are indicated in the last column of each table. *Art* and *Ven* stand for arterial and venous blood respectively, *cap* means that the blood was saturated with 40 mm of  $\text{CO}_2$  while *cont* means that the blood was analyzed as drawn, without exposure to the air.

The patients in the first part of table 7 were suffering from nervous conditions that presumably would not affect the plasma proteins. The range of variation does not differ appreciably from that of the normals shown in the preceding table.

In the second part of the table appear four patients with arteriosclerosis and hypertension without evidences of functional disturbances of heart or kidney. With one exception these patients showed normal plasma proteins. No. 22158, at the time of the first observation had very high proteins in his plasma. At this time, three days after his admission to the hospital, he was in coma, difficult to feed, and his fluid intake was small. Five days later, with some improvement, the plasma proteins, hemoglobin and hematocrit had fallen together. Calculations of the relative plasma volumes made on the basis of the hemoglobin and hematocrit figures show that the blood plasma had been diluted 11 per cent. Similar calculations based on

TABLE 7

Case number	Oxygen capacity	Cell volume	Plasma proteins*	Character and treatment of blood	Remarks
Section 1					
12016	vols per cent 22 4	vols per cent 47 0	per cent 7 71	Ven Cap	Neurasthenia Wassermann test positive
29189	20 9	45 1	6 94*	Ven Cap	Syphilis of central nervous system
15004	20 2	43 4	7 94*	Ven cap	Psychoneurosis
10875	20 6	43 7	7 69*	Ven cap	Neurasthenia Wassermann test positive
10859	18 2	39 9	7 17*	Ven cap	Hysteria
26871	17 3	38 9	6 73*	Ven cap	Ghoma of left parietal lobe
26451	21 1	48 0	7 52*	Ven cap	Cataract. Chronic frontal sinusitis

## Section 2

26362	16 4	36 5	7 31	Art cont	Arteriosclerosis, hypertension, old hemiplegia
22158	22 1	48 3	8 60	Ven cap	Arteriosclerosis, hypertension, cerebral hemorrhage, hemiplegia and coma
	20 7	46 1	7 81	Ven cap	5 days later
6657	18 5	41 1	7 61	Ven cap	Hypertension, obesity
18081	29 3	39 1	6 54	Art cont.	Arteriosclerosis, hypertension

## Section 3

10702	18 7	40 8	7 00*	Ven cap	Methyl alcohol poisoning In convulsions Has received large amounts of sodium bicarbonate
29658	20 6	46 4	7 97	Ven cont	Obstructed ventral hernia Intestinal obstruction
9420	21 6	44 7	7 81*	Ven cap	Acute colitis Moderately severe
10495	17 9	35 5	6 70*	Ven cap	Acute alcoholic gastritis
26690	10 9	24 4	5 52	Art cont	Arteriosclerosis with hypertension Carcinoma of the ascending colon with intestinal obstruction
22798	15 90	34 6	4 89	Art cont	Extensive burns of trunk Has been given enormous amounts of water and salt solution by every means until generalized subcutaneous edema developed

TABLE 7—Continued

Case number	Oxygen capacity	Cell volume	Plasma proteins	Character and treatment of blood	Remarks
Section 3—Continued					
26515	17 0 <i> vols  per cent</i>	54 7 <i> vols  per cent</i>	6 30 <i> per cent</i>	Ven cont.	Toxic vomiting of pregnancy Cellulitis—no edema—fluid intake by parenteral routes large
Section 4					
18725	20 1	43 3	8 63	Ven cap	Hemorrhagic pachymeningitis Bronchopneumonia. Patient stuporous and having frequent convulsions on the day of the examination
33564	15 3	36 0	7 03	Ven cont.	Chorea major Patient in coma
15712	21 2	43 6	7 66	Ven cap	Carbon monoxide poisoning Influenza. Patient in coma
15703	20 1	42 1	6 62	Ven cap	Lobar pneumonia. Fourth day
10744	16 2	40 9	6 77	Ven cap	Acute tracheitis, broncho-pneumonia, fibrous pericarditis, one day before death
26343	15 9	38 2	7 01	Ven cap	Acute mediastinitis
Section 5					
5196	18 4	39 9	6 73	Ven cap	Arsphenamine poisoning with central necrosis of the liver
1536	10 4	24 9	7 53*	Ven. cap	Seven year old boy with anemia and unexplained jaundice
5238	21 4	50 1	7 40	Ven cap	Atrophic cirrhosis of liver Moderate ascites Hypertension
34777			5 52	Ven cont.	Female, aged 53, with obstruction of the common bile duct and an ascending bile duct infection. Extremely toxic. Blood non-protein nitrogen 80 mg. in 100 cc. Intense jaundice

\* Non-protein nitrogen was not determined on these samples, but the values for total nitrogen, in terms of per cent protein, were reduced by 0 19, corresponding to a blood non-protein nitrogen of 30 mg per 100 cc.

plasma protein values show a 10 per cent dilution. The high protein of the first observation was apparently merely a product of blood concentration, possibly due to his low fluid intake, and was not directly referable to the diseased condition. Hypertension and arteriosclerosis in themselves seem to have no specific effect on the plasma proteins.

In the third portion of the table is a group of miscellaneous cases. Nos. 10702, 9420, 10495 and 26515 were chosen for study in the hope that they would show dehydration from loss or deprivation of fluids. All of them had, however, received adequate fluids before the time of the examination. No. 29658, an old woman with an obstructed ventral hernia who had been vomiting continuously for more than 24 hours, does show some elevation of the plasma proteins with a high normal hemoglobin. Unfortunately no other observation was made on this patient.

No. 26690 had a carcinoma of the ascending colon which had become obstructed shortly before admission. At the time of the blood examination the intestinal obstruction had been overcome, vomiting had ceased and large amounts of fluid had been given the patient. He had, however, had a profuse hemorrhage from the bowel and appeared exhausted and toxic. How far the hemorrhage may be held responsible for the low proteins and how much dilution of the blood had been caused by the forcing of fluids it is impossible to say.

No. 22798, a middle aged man who had received extensive burns of the face, trunk and extremities was not examined until a few days before death. For four days before the blood study his fluid intake by mouth and by hypodermoclysis had been from 4500 to 9000 cc. At the time of the examination he had a moderate generalized edema and a liquid diarrhea. His hemoglobin, which had been determined daily by the Cohen and Smith method, had fallen from 140 per cent (Haldane scale) to 45 per cent. Although no other observations of the proteins were made it seems more than likely that the low value found in this case was largely due to dilution of the blood. It should be added that none of the patients of this series had blood non-protein nitrogens above 50 mg per 100 cc of blood.

In the fifth section of table 7 appear four observations on subjects with diseased conditions of the liver, all but one of which have normal

plasma proteins Atchley, Loeb, Benedict and Palmer (1) noted a reduction of serum protein in three patients with cirrhosis of the liver and ascites That such a reduction is not characteristic of this condition is evident from case no 5238 In one case with low proteins, no 34777, the reduction may well have been referable to the general state of intoxication and shock, and not to the liver injury

Rowe (11) and others have found that in acute infectious conditions there is an increase of the globulin of the serum, associated with a

TABLE 8

Case number	Oxygen capacity	Cell volume	Plasma protein	Character and treatment of blood	Remarks
	<i> vols per cent</i>	<i> vols per cent</i>	<i> per cent</i>		
18859	3.57	11.0	6.97*	Ven cap	Adenomyoma of uterus Secondary anemia
8169	2.65	5.4	5.89*	Ven cap	Pernicious anemia Slight generalized, subcutaneous edema
	2.89	6.1	6.57*	Ven cap	Same patient, 9 months later Edema is again present
9734	4.23	12.8	7.49*	Ven cap	Secondary anemia Cause undetermined
22272	4.90	18.6	3.68	Ven cont.	Advanced pulmonary tuberculosis General anasarca
22114	3.71	9.4	5.52*	Ven cap	Adenoma of uterine cervix Slight edema of ankles
33106	3.61		5.42*	Ven cont.	Pernicious anemia. General anasarca
10210	10.4	23.0	6.95*	Ven cap	Pernicious anemia
22780	28.7	65.4	7.23*	Ven cap	Polycythemia
	27.6	61.0	6.34	Ven cont.	Same patient, 2 weeks later

\* Non protein nitrogen was not determined on these samples, but the values for total nitrogen, in terms of per cent protein, were reduced by 0.19, corresponding to a blood non-protein nitrogen of 30 mg. per 100 cc.

reduction of the albumin, with the result that the total proteins are normal or slightly reduced In the fourth section of table 7 are shown a few cases with severe infections Nothing can, of course, be said about the relation of globulin to albumin in these plasmas, but the total protein, at any rate, shows no tendency to reduction In many of these cases the diminution may have been masked by a concentration of the blood caused by inadequate fluid intake, but the hemo-

globin values give no indication of such a concentration. In no 10744 such an explanation is certainly unsatisfactory because the patient was almost anuric and had received large amounts of fluid by mouth and subcutaneously. The increase of fibrinogen which is such a constant finding in pneumonia and other infectious conditions would of course tend to make the plasma proteins relatively higher than the serum proteins.

Low serum proteins have been reported in severe anemias by Kahn and Barsky (7) and others. In eight observations on seven patients shown in table 8 low proteins were found in the plasma five times. It is interesting that in four of these five instances the patients presented subcutaneous edema. The association of edema and low proteins appears, however, to be merely coincidental because one of the same patients, no. 8169, on a second occasion had normal proteins in the presence of edema. The low proteins, furthermore, do not seem to depend on the presence, severity or type of anemia. In one case of polycythemia the plasma proteins were normal.

#### SUMMARY

1 Determination of the proteins in normal oxalated plasma gives values appreciably lower than those obtained from the analysis of serum, in spite of the fact that the latter lacks fibrinogen. This is due to the shrinking of the blood cells produced by oxalate.

2 The changes in carbon dioxide and oxygen tension of the blood encountered under ordinary conditions cause demonstrable but inconsiderable changes in the plasma proteins.

3 The production of venous stasis results in an increase of the proteins in the venous plasma of the affected part which is due to a transfer of water from the blood to the tissues.

4 Exercise may cause concentration of the venous plasma that is reflected in an increase of the plasma proteins.

5 Under certain conditions the exchange of water between the blood and the tissues in the capillaries may be so greatly accelerated that the concentration of proteins in arterial and in venous plasma may differ to a significant degree.

6 The degree of variation of the plasma proteins in a group of five normal individuals taken under various conditions was considerable.

and agreed with the reports of other observers. The degree of variation observed in a single individual over a period of two years was very slight. The same individual, observed under standardized conditions over a shorter period presented even smaller variations in the proteins of his venous plasma. Seven patients with neurologic or psychopathic disorders which presumably would not affect the plasma proteins showed values similar to those found in the normal group.

7 Arteriosclerosis and hypertension in themselves appear to have no effect on the plasma proteins. One patient with cerebral hemorrhage, in coma, had high proteins in his plasma. As his condition improved proteins, hemoglobin and cell volume fell simultaneously. The high cell volume and proteins were evidently due to a concentration of the blood, possibly caused by an inadequate fluid intake.

8 The administration of excessive fluids to a patient with severe burns resulted in the production of hydremia with a consequent reduction of the plasma proteins. Edema also developed.

9 In a series of five cases with severe acute infections the plasma proteins were normal or slightly elevated.

10 The effects of factors which may alter the water content of the blood must not be neglected in the interpretation of values of the proteins of the plasma.

11 Normal plasma proteins were encountered in three patients with diseases of the liver, in one instance in the presence of ascites.

12 Although low plasma proteins appeared four times in seven observations on patients with profound anemia, there seemed to be no relation between the reduction of the proteins and the presence, severity or type of anemia. Edema, which so commonly occurs in these cases, is not necessarily associated with a diminution of the plasma proteins.

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- 5 Under certain conditions the exchange of water between the blood and the tissues in the capillaries may be so greatly accelerated that the concentration of proteins in arterial and in venous plasma may differ to a significant degree.

- 6 The degree of variation of the plasma proteins in a group of five normal individuals taken under various conditions was considerable.

# THE PLASMA PROTEINS IN RELATION TO BLOOD HYDRATION

## II IN DIABETES MELLITUS

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Rowe (17), in 1917, reported observations of the serum proteins of 10 patients with diabetes. The only remarkable thing was the extreme variability of the findings. Both high and low values were obtained. In the majority of instances albumin and globulin preserved their normal proportions, but in some instances globulin appeared relatively high. Subsequent observers have confirmed Rowe and the general opinion appears to be that there is nothing characteristic about the serum protein level in diabetes.

In table 1 are shown the results of 52 observations of the plasma proteins of 31 patients with diabetes mellitus of varying degrees of severity, studied at different stages of the disease.

### EXPERIMENTAL PROCEDURE

Whenever there is no note to the contrary, the blood was taken in the morning, before the patient had breakfast and the morning dose of insulin. The blood was withdrawn in a syringe and coagulation prevented by the addition of enough neutral potassium oxalate to make an 0.2 per cent oxalate solution in the blood. The treatment of the blood is indicated in column 6 of the table. Those specimens marked *cont* were obtained without stasis and immediately placed over mercury in a blood sampling tube of the type described by Austin, Cullen, Hastings, McLean, Peters and Van Slyke (1). Usually venous blood (*ven*) was employed, but occasionally arterial (*art*) was used instead. In specimens indicated as *cap* the blood was withdrawn without precautions against air contact and brought into equilibrium with 40 mm. of CO<sub>2</sub> in the air at 38°C. by the method previously described (16) before it was placed in the sampling tube over mercury. From the mercury sampling tube part of the blood was transferred to a centrifuge tube

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## Section 2

20387		57 2	18 1	38 2	5 58	Ven cap	Female, aged 13, with mild diabetes. On the day of examination she showed a transitory glycosuria and began a rather extraordinary diuresis that lasted 3 days. Urine shows a moderate amount of glucose but no acetone.
10629	291	47 8	18 7	11 5	7 18*	Ven cap	Female, aged 62, with apparently mild diabetes aggravated by a fracture of her skull. Urine contains large amounts of sugar and acetone.
29127	167	57 1	18 1	12 6	6 72	Ven cap	Female, aged 50, with mild diabetes aggravated by presence of lobar pneumonia. Blood taken the morning after admission when urine contained large amounts of sugar but no acetone.
15921	112	43 5	11 3	32 3	6 25	Ven cap	Female, aged 65, with diabetic gangrene of foot, aggravating mild diabetes, after amputation.
10888	311	71 2	17 8	38 3	6 17	Ven cap	Female, aged, 53, with chronic and acute arthritis. Temperature at time of examination, 99.6°, arthritis symptoms relieved. In spite of high blood sugar urine is sugar free.
15175	214	53 1	20 9	11 9	7 25	Ven cap	Female, aged 60, with mild diabetes complicated by a fractured patella and lacerated wounds of the forehead. At time of examination, the second day after admission, urine showed large amounts of sugar but no acetone.
34618	309	59 9	17 7	34 2	6 10*	Ven cont	Female, aged 33, with moderately severe diabetes, the morning after admission to the hospital. Urine contains considerable sugar, acetone and diacetic acid. Patient is thin, but not extremely emaciated and not dehydrated.
1911	123	34 2	19 7	38 3	7 35*	Ven cap	Male, aged 17, with severe diabetes and diabetic acidosis, at time of admission to hospital. Marked emaciation, polydipsia and polyuria. Urine shows moderate amount of sugar and considerable acetone.

TABLE 1

Case number	Blood sugar	Plasma CO <sub>2</sub>	Oxygen capacity	Cell volume	Plasma proteins	Character of blood sample	Remarks
Section 1							
	mgm. per 100 cc	vols per cent	vols per cent	vols per cent	per cent		
19043	293	52.6	21.4	43.8	6.19	Ven cap	Male, aged 62, with mild diabetes admitted for cataract operation. Blood taken the day after admission.
5105	231	53.3	21.3	45.6	6.91*	Ven cap	Male, aged 38, with mild diabetes. Blood taken day after admission.
26903	215	53.0	23.2		7.21	Ven cap	Male, aged 56, with mild diabetes, arteriosclerosis and hypertension. Blood taken 6 days after admission.
12562	139	48.9	20.0	43.0	7.41	Ven cap	Female, aged 45, with mild diabetes and an enlarged liver. Blood taken the morning after admission.
77914	141	59.3	20.4	41.5	6.81*	Ven cap	Male, aged 44, an outpatient with alimentary glycosuria or mild diabetes. Blood taken before breakfast.
10919	132	55.5	22.1	44.3	7.31*	Ven cap	Male, aged 40, brother of the preceding patient, with mild diabetes and central nervous system syphilis. Blood taken the morning after admission.
10801	411	45.1	19.5	41.7	6.60	Ven cap	Female, aged 66, with mild diabetes, arteriosclerosis, hypertension and paralysis agtans. Blood taken 3 days after admission.
16727	147	57.5	20.5	44.6	7.36*	Ven cap	Male, aged 55, with mild diabetes and hypertrophic spondylitis.
15733	319	63.8	17.8	38.5	5.98	Ven cap	Female, aged 64, with mild diabetes, arteriosclerosis and hypertension. Had suffered a small cerebral hemorrhage the day before admission, and had also vomited a few times. Blood taken the morning after admission.
26143	110	56.0	22.0	45.5	6.94	Ven cap	Male, aged 50, with mild diabetes and chronic frontal sinusitis.

29751	231	64 0	19 2	12 5	6 20	Ven cont	Same patient 2 days later, after insulin treatment has gained 5 pounds on maintenance diet only Urine showed only trace of sugar and moderate amount of acetone
		43 9	20 1	44 0	7 91*	Ven cont	Male, aged 37, with severe diabetes, complicated by otitis media, examined the morning after admission Appears emaciated and dried up Urine contains large amounts of sugar and acetone
	113	85 0	17 7	10 7	6 60*	Ven cont	Same patient 2 days later, after insulin treatment Patient has gained 5 pounds on maintenance diet only Urine shows only trace of sugar and acetone
18467	332	59 2	15 3		6 72*	Art cont	Male, aged 50, with moderately severe diabetes complicated by an acute respiratory infection, examined the morning after admission Only slight evidences of desiccation Urine contains large amount of sugar but no acetone
	133	55 1	16 6		6 26*	Art cont	The same patient later in the day, after he had received 10 units ofletin
15120	419	50 6	20 1	46 2	7 14	Ven cap	Female, aged 57, with mild diabetes, complicated by lobar pneumonia, examined at time of admission Semicomatose, dyspneic, with labored breathing and cyanosis Consolidation of right lower lobe Urine contains large amount of sugar and considerable acetone
	271	52 1	22 3	46 8	7 13	Ven cap	The same patient next day, afterletin treatment Urine free from sugar and acetone General condition seemed improved, but patient died later in the day of cardiac failure
29061	308	16 8	22 5	15 8	8 37	Ven cont	Female, aged 56, with mild diabetes mellitus, 18 hours before admission patient had a slight cerebral hemorrhage The next day she became stuporous and her respirations increased She vomited a few times Examination of blood was made at time of admission, the night of January 28, when patient was comatose, and urine contained large amounts of sugar and acetone

TABLE 1—Continued

Case number	Blood sugar	Plasma CO <sub>2</sub>	Oxygen capacity	Cell volume	Plasma proteins	Character of blood sample	Remarks
Section 2—Continued							
15267	811	52.8	19.2	37.4	6.28*	Ven cap	Male, aged 21, with severe diabetes. Blood taken at time of admission. Urine showed large amount of sugar and moderate acetone. Patient emaciated and dry. Blood pressure systolic 90, diastolic 60.
10572	290	63.3	17.2	36.2	5.75*	Ven cap	Male, aged 46, with moderately severe diabetes. At the time of the blood examination, November, 1922, on inadequate diet, urine free from sugar and ketones. Slight edema of ankles.
	271	45.1	17.5	41.4	7.68*	Ven cap	The same patient a year later, October, 1923, the morning after his admission to the hospital for an acute upper respiratory infection. Urine contains large amounts of sugar and acetone.
18067	1,000	16.5	23.3	44.7	5.45	Ven cap	Female, aged 37, with acute diabetes. 2 days before admission she was seized with severe abdominal pain and vomiting, which persisted till admission. Examination was made at time of admission when patient was in diabetic coma. She appeared extremely dehydrated. Blood pressure systolic 80, diastolic 45. Urine contained large amounts of sugar and acetone.
Section 3							
26461	263	56.9	20.4	44.3	6.37	Ven cont	Male, aged 33, with moderately severe diabetes, examined in the morning after admission. At this time looked desiccated and had polyuria and polydipsia. Urine contained large amounts of sugar and acetone.

15096	712	51 1	22 1	17 2	7 29	Ven cap	Female, aged 17, with mild diabetes and renal tuberculosis who had recently developed an influenzal pneumonia. She had been vomiting almost continuously for 4 days and had received large quantities of sodium bicarbonate. Examination made at time of admission, the night of December 11, 1922, when patient was stuporous, but breathing quietly. She complained of thirst and appeared dehydrated, but presented a slight edema of ankles. Urine contained large amounts of glucose and acetone.
	216	76 5	21 3	15 1	5 77	Ven cap	The same patient two days later, December 13, after insulin treatment, without alkali. General condition improved, but vomiting and hiccough continue. Urine contains no sugar nor acetone.
	297	51 1	18 1	10 1	6 30	Ven cap	The same patient one month later, January 11, 1923, shortly before discharge from hospital. Urine contains no sugar nor acetone and patient is receiving an adequate diet without insulin.
22350	616	13 5	20 1		8 02	Ven cont	Female, aged 50, with severe diabetes 4 days before admission. Patient was seized with severe abdominal pain and vomiting which persisted until admission to hospital. Examination made at time of admission the night of November 19, 1923, when patient was stuporous. She appeared extremely dehydrated. Urine contained large amounts of sugar and acetone.
	311	33 7	20 1		7 15*	Ven cont	The same patient the next morning, November 20, after insulin treatment, saline hypodermoclysis and sugar solution by mouth. Respirations quieter, mental condition clear. Urine shows considerable sugar and moderate acetone.



TABLE 1—Continued

Case number	Blood sugar	Plasma CO <sub>2</sub>	Oxygen capacity	Cell volume	Plasma proteins	Character of blood sample	Remarks
Section 3—Continued							
15670	mgm. per 100 cc	vols per cent	vols per cent	vols per cent	per cent		
	142	41 6	21 0	42 4	8 07*	Ven cont	The same patient the next morning, January 29, after insulin and forcing fluids with sugar. Respirations quiet. Urine contains considerable sugar, but little acetone.
	229	49 3	19 0	40 6	6 20*	Ven cont	The same patient one day later, January 30, improvement continues. Urine shows some sugar, but practically no acetone. Patient has retained considerable fluid.
	241	60 8	18 4	38 2	5 85*	Ven cont	The same patient one week later, February 6. Urine free from sugar and acetone on an adequate diet with insulin. Consciousness has returned, but she has a definite aphasia.
	441	30 9	13 8	27 8	5 36*	Ven cap	She also has an irregular pulse and the electrocardiograph reveals evidences of coronary disease.
	313	62 1	15 2	30 9	5 03*	Ven cap	Male, aged 26, with severe diabetes, emaciated and dehydrated. Urine contains large amount of sugar and acetone. Examination at time of admission, February 12, 1923.
	273	53 8	14 2			Ven cap	The same patient 10 days later, February 22, much improved. He has, however, gained 37 pounds and has developed general anasarca. His urine contains a trace of sugar, but no acetone.
							The same patient after 10 more days, March 2, has lost 27 pounds and his weight is diminishing steadily although his diet has increased. No signs of edema remain. Urine contains a trace of sugar but no acetone.

and the plasma separated. The latter was then removed to a second sampling tube over mercury and from this samples were taken for analysis. The remainder of the blood was utilized for the determination of cell volume and for other purposes of no immediate importance in this connection.

For the estimation of cell volume an hematocrit of the Daland type manufactured by the International Instrument Company for their centrifuges was employed. Carbon dioxide was determined by the method of Van Slyke and Stadie in a water-jacketed machine calibrated in 0.01 cc. For the oxygen capacity a technique devised by Lundsgaard and Neill<sup>1</sup> which permits the saturation of the blood in the Van Slyke apparatus, was used. In order to reduce the amount of blood required the oxygen capacity was frequently determined not on the whole blood, but on the cell residue. The centrifuge tubes in which the plasma was separated were especially prepared with contracted necks. After the cells had been whirled down the upper level of the plasma was carefully marked. After the plasma had been removed the tube was filled to the mark with 0.9 per cent NaCl solution, the cells were well mixed with the saline, and the resulting emulsion was employed for the determination of oxygen capacity. Values thus obtained agree with those obtained directly from whole blood. (If water is used for the dilution instead of saline the resulting solution becomes so viscous that it is unmanageable.) For the total nitrogen of the plasma an ordinary macro-Kjeldahl technique was employed. Non protein nitrogen was determined on the whole blood by the Folin-Wu method (7), except that distillation and titration with 0.02 N alkali was substituted for direct Nesslerization. In some instances, indicated by an asterisk in the table, non protein nitrogen was not determined. In these cases 0.19 per cent (corresponding to 30 mg of nitrogen per 100 cc) was subtracted from the values of the total nitrogen in terms of protein. This was only done when there was a reasonable certainty that the blood non-protein nitrogen would prove normal. In no case can this omission introduce any significant error. It is, of course, not strictly proper to use blood non-protein nitrogen values for plasma. Presumably the latter would have proved somewhat less. The necessity for economy of blood made direct determinations on plasma impracticable.

In some of the earlier experiments no special effort to avoid venous stasis was practised in the collection of *cap* specimens. Partly this was due to a failure to appreciate the importance of such precautions, partly because the blood was intended more particularly for other purposes and the studies of plasma proteins were only incidental. This invalidates none of the important experiments. In the earliest observations the plasma used for protein determinations was made up from all the remnants left from other work on the blood specimens and portions of it were even derived from blood that had been exposed to the air. This probably introduced no serious errors but must be taken into consideration in at least one case, no 15096.

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<sup>1</sup> Personal communication

TABLE 1—*Concluded*

Case number	Blood sugar	Plasma CO <sub>2</sub>	Oxygen capacity	Cell volume	Plasma proteins	Capacity of blood sample	Remarks
Section 3—Concluded							
34611	<i>mgm. per 100 cc</i> 349	<i>vols per cent</i> 47.8	<i>vols per cent</i> 17.0	<i>vols per cent</i>	<i>per cent</i> 5.94*	Ven cont	The next morning, November 21 Improvement has continued under treatment with insulin and carbohydrate fluids Urine contains sugar, but no acetone
	249	73.8	16.9		6.24*	Ven cont.	6 days later, November 27 Patient receiving an adequate diet and, with insulin her urine remains free from sugar and acetone
	242	67.4	16.7		6.73	Ven cont	3 weeks later, December 17 Patient continues well on adequate diet with insulin and urine remains free from sugar and acetone
	315	41.4			6.13*	Ven cont	Male, aged 19, just recovering from severe diabetic toremia precipitated by a gastric upset, with vomiting Urine contains large amounts of sugar, acetone and diacetic acid Patient appears dehydrated
	341	65.6			5.45*	Ven cont	Same patient, 5 days later, on a comparatively low diet, receiving insulin Urine contains no sugar, acetone nor diacetic acid

second occasion he had been taking insulin for some time, with excellent results, and was admitted to the hospital only because he had developed a cold and a slight pleurisy that was attended by an exacerbation of diabetic symptoms and the appearance of large amounts of sugar and acetone in the urine. With this he lost weight very rapidly and, at the time of the examination, appeared distinctly dehydrated. It will be noted that the plasma proteins are near the upper limit of normal. The hemoglobin does not, however, appear elevated.

With one other exception the values observed vary only within normal limits and the variations bear no relation to the level of blood sugar, carbon dioxide or hemoglobin. No. 18067 was an unusual case in every respect. She was admitted in diabetic coma within 48 hours after the onset of an acute attack of abdominal pain attended by persistent vomiting. No previous history of diabetes could be obtained. Blood was taken immediately after she entered the hospital when she was in deep coma, with typical respirations of acidosis. She appeared extremely desiccated, her pulse was weak and rapid and her systolic blood pressure only 80 mm. With insulin she was brought out of coma, and the respirations were temporarily quieted, but she sank back into her original state with extreme rapidity. After her admission to the hospital she passed very little urine and the administration of fluids by mouth and by hypodermoclysis resulted only in the development of edema. She died within 72 hours after her admission to the hospital.

The high hemoglobin at once suggests a concentration of the blood from loss of water and this seems reasonable in view of the vomiting and the overventilation. On the other hand concentration can offer no explanation for the low proteins. In fact no mere alteration of blood water can explain the paradoxical findings. There was no evidence of any preexisting condition that could have determined such extremely low proteins. The immediate disease might have caused a specific destruction of the plasma proteins, but the duration of symptoms was so short that this seems unlikely. It is far easier to believe that proteins had passed from the blood. White and Erlanger (19) found that in surgical shock and allied conditions, although the cellular elements of the blood became concentrated by

Blood sugar was determined by the method of Folin and Wu (8) except in a few of the earlier studies when Benedict's modification of Lewis and Benedict's method (2) was used

### OBSERVATIONS

The 13 observations in the first section of table 1 are from mild and for the most part uncomplicated cases of diabetes. The majority were made before breakfast the morning after the patients were admitted to the hospital, and, therefore, while glycosuria still persisted and before dietary treatment had been well started. None of these patients showed evidences of serious dehydration or any considerable loss of weight. The plasma proteins are, in all but two of the twelve observations quite normal. The two exceptions, nos 15733 and 20387 both had rather low proteins. The reduction in 15733 is slight, 5.98, and there were so many complicating factors in the case that it is impossible to ascribe the fault to diabetes. It is unfortunate that only one observation was made on no 20387. The low protein was quite unexpectedly encountered in the course of an experiment in which the blood was being used for control purposes only. In reviewing the case afterwards it was found that on the day of the blood examination the patient had a transitory glycosuria and an extraordinary diuresis that persisted for 3 days. Of course it is impossible to connect these phenomena with the low proteins directly in the absence of other examinations of the same individual. It may be of some significance that the lowest proteins in this series were associated also with the lowest hemoglobin.

The 10 observations of section 2 represented single examinations of patients with diabetes of inherently greater severity or aggravated by some complicating condition. Although no 10572 was studied on two occasions it is impossible to compare the two observations because an interval of almost a year had elapsed between them. On the first occasion he came to the hospital extremely malnourished, on a low diet. Extreme dietary reduction was necessary to render him aglycosuric and to overcome an initial acidosis. Under these circumstances, as is so often the case, the early part of his recovery was attended with the development of some edema. It was during this edematous period that the low proteins were found. On the

It is hard to avoid the feeling that these rapid alterations of plasma protein that occur in simple cases are merely expressions of changes in the state of hydration of the blood. The fact that the calculations based on hemoglobin and cell volumes do not agree exactly with those based on plasma protein values does not refute such a theory. Perfect agreement could hardly be expected in view of the profound disturbances of circulation that must accompany conditions of diabetic intoxication and may affect the number of cells circulating in the peripheral blood. Regeneration and destruction

TABLE 2

Case number	Relative plasma volume calculated from		Remarks
	O-capacity and cell volume	Plasma proteins	
26461	1 00	1 00	At time of admission
	1 10	1 03	2 days later
29754	1 00	1 00	At time of admission
	1 20	1 19	2 days later
15120	1 00	1 00	At time of admission
	0 89	0 96	The next morning
29061	1 00	1 00	At time of admission, January 28
	1 14	1 04	The next morning, January 29
	1 30	1 35	One day later, January 30
	1 39	1 43	One week later, February 6
15670	1 00	1 00	At time of admission
	0 87	1 07	10 days later
15096	1 00	1 00	At time of admission
	1 09	1 26	2 days later
	1 38	1 16	1 month later

of the proteins themselves must go on and may share in the general metabolic disturbance that exists. With the recognition of the importance of hydration as a factor, however, little has been gained. The production of appreciable changes in the water content of the blood in normal individuals is difficult. To effect a plasma dilution amounting to 30 per cent is almost impossible.

Widal, Abram, Weill and Laudat (20) have noted similar changes in the concentration of the proteins in the serum of diabetic patients with acidosis after the administration of insulin and they also interpret the diminution of protein as an expression of blood dilution.

loss of water to the tissues, the plasma proteins fell. The low blood pressure and the clinical picture in this case is quite suggestive of "shock" and it is not surprising, therefore, to find an inspissation of the blood with low plasma proteins. If this explanation of the findings is correct the protein change can not be referred directly to the diabetes but must be ascribed to the condition that precipitated the acute crisis. No clue to this underlying condition could be found and autopsy was refused.

On each of the 9 cases in section 3 two or more studies were made at comparatively short intervals and opportunity is therefore given to study the mechanism of some of the variations that occur in different stages of the disease. The first 2 patients, nos 26461 and 29754, had diabetes of moderate severity, in the latter case complicated by an otitis media. Both had typical diabetic symptoms, and on admission appeared dehydrated. They had been losing weight rapidly and showed marked glycosuria and ketonuria. In each case the administration of insulin and adequate amounts of fluids resulted in a striking increase in weight with a simultaneous fall in both hemoglobin and plasma proteins. In table 2 the relative plasma volumes, calculated from hemoglobin and cell volume and from plasma protein, are compared. In both instances the changes calculated by the two methods are in the same direction, in the second case they are also of the same magnitude. One can not escape the feeling that water exchange is the predominant factor behind such alterations and that the preliminary dehydration and subsequent restoration of the normal fluid content of the body are reflected in the protein values. The fourth case, no 15120, presents a different picture. The patient, an elderly woman with diabetes and a complicating pneumonia was in a desperate state at the time of admission. Dehydration was quite noticeable and the administration of fluids by mouth difficult. Although with iletin it proved possible to overcome the glycosuria and ketonuria, the overventilation, temperature and inadequate fluid intake resulted probably in a further negative water balance which is associated with an increase of plasma proteins, hemoglobin and cell volume. The changes in nos 29061, 15096 and 22350 seem to be of the same nature and in the first and last of these three cases the high initial values, 8.37 and 8.02 are indicative of a very definite concentration of the blood.

alkaline reserve, the state of hydration apparently bears no relation to the absolute level of plasma  $\text{CO}_2$ . In only one or two cases was the  $\text{CO}_2$  of the plasma above the upper normal level and edema occurred frequently in the presence of a normal  $\text{CO}_2$ . From a clinical standpoint only, the conditions that usually result in edema in diabetes can be fairly well defined. It usually occurs in poorly nourished individuals who are receiving an inadequate diet, but show no reduction in the plasma bicarbonate. It is especially apt to develop during or just after recovery from a severe attack of diabetic toxemia. Before the advent of insulin it was more common than it is at present. The development of acidosis or the administration of acidosis producing drugs results in rapid diuresis. In the absence of such therapy the edema disappears when the diet is increased to meet the demands of the individual. This is well illustrated by case no 15670. At the time of the second blood examination he had developed general anasarca. At that time he was receiving only 1340 calories without insulin and had moderate glycosuria. After the addition of enough insulin to clear the glycosuria, the edema disappeared as rapidly as it had come. No 10572 delivered his edema as soon as he was given a maintenance diet and insulin.

This nutritional factor plays a part in the development of edema that appears to be independent of the acid-base equilibrium, or at least of the level of the plasma or blood carbon dioxide. It may be some of the acid-base elements other than bicarbonate that is at fault. Peters (15), Stillman, Van Slyke, Cullen and Fitz (18), Cullen and Jonas (21) and most recently Bock (3) have noted that after recovery from diabetic acidosis the plasma bicarbonate rises rapidly, while the alveolar carbon dioxide tension remains low. If the alveolar carbon dioxide is the same as that of the arterial blood such a discrepancy can only result in an uncompensated alkalosis. This Bock and his coworkers found in some of their cases. We have also noted a tendency on the part of some of these patients to develop a mild grade of uncompensated alkalosis during the period of recovery.

Gamble, Ross and Tisdall (9) suggest that the water balance is more strictly determined by the level of the alkaline metals in the body and that a retention of these elements is attended by a diminished fluid output. To test this hypothesis we have analyzed the



They could not, however, connect this directly with the water balance nor with the glycemia, the acidosis nor the assimilation of carbohydrates. In those of our cases in which rapid dilution occurred there is a definite retention of fluids and increase of weight as the protein falls. That the administration of fluids alone is not capable of producing such an effect is, however, well illustrated by case no 15096 who was admitted with an edema. It is also clear from this case that edemas which occur in the course of diabetes are not invariably associated with low plasma proteins and blood dilution.

Gamble, Ross and Tisdall (9) have shown that fasting results in a loss of water from the body and that this dehydration is one of the effects of acidosis and depletion of base. The well known diuretic effects of ammonium chloride and calcium chloride have been shown by Haldane (10) to depend also on the production of an acidosis. It has repeatedly been demonstrated (5) that the edemas which occur in the course of diabetes can be rapidly delivered by the administration of these acid producing salts and that edema is prone to develop after the administration of sodium bicarbonate. Oehme (14) has made the statement that all conditions that tend towards the production of acidosis result in diuresis, and, vice versa, any increase in the alkalinity of the blood leads to a retention of fluid. At first sight it seems as if in these studies, also, dehydration was associated with acidosis and retention of fluid with the restoration of the normal alkalinity of the blood.

No 18067 of section 2, mentioned above, is apparently an exception to the rule, but this must not be given too much weight because, as we said above, she was exceptional in every respect. When fluid balance is considered the case seems comparatively clear. From the standpoint of blood hydration, however, frequent inconsistencies appear. For instance, no 15096, at the time of admission, had a normal plasma  $\text{CO}_2$  and an edema, at the same time both hemoglobin and plasma proteins were high, indicating a concentration of the blood. Apparently the hydration of tissues and blood may be dissociated in diabetes just as they have been found to be in nephritis and in cardiac disease.

Although tissue dehydration is in most instances associated with acidosis and retention of fluid with the restoration of the normal

or the other metabolic disorders associated with diabetic toxemias that determines the tissue dehydration is well illustrated in no 15096. This patient had received large doses of alkali before admission and had also vomited large amounts of food and fluid, possibly with the loss of considerable chloride. The consequence was that in spite of the presence of an enormously high blood sugar, marked ketonuria and all the symptoms of impending diabetic coma except the hyperpnea, her plasma  $\text{CO}_2$  content proved normal. Under these circumstances she developed an edema during the diabetic intoxication.

Although it is possible to explain the *changes* that occur in the plasma proteins over short periods of time on the basis of alterations of blood hydration in the majority of cases, in a few the facts do not seem to permit such an explanation. For example in no 18467 the oxygen capacity rose in the course of a few hours from 15.3 to 16.6, while the proteins fell from 6.72 to 6.26. Cell volumes were not determined in this case, but it is inconceivable that the cells could have shrunk enough to account for such a discrepancy. Such occasional exceptions do not seem to us to invalidate our explanation. It would be extraordinary indeed if hemoglobin, cells and proteins were always proportionally distributed in all parts of the circulating blood. The matter can be definitely decided only by the simultaneous application of some other method for the direct determination of plasma volume.

Comparisons of proteins and hemoglobin made at intervals of more than two days in most instances show no parallelism. The methods are inapplicable to studies over long periods because of the play of the forces that determine regeneration and destruction of proteins and blood cells. In case 15096, for instance, at the time of the second examination hemoglobin, cell volume and plasma proteins had all fallen together, one month later at a third examination the hemoglobin and cell volume were still lower, but the plasma proteins had risen to the normal level. No 22350 shows a similar course of events. One gains the impression that the high proteins found in the first examination were due to the extreme dehydration of a blood that really contained less protein than normal. Relieving the acute condition results in the restoration of the normal plasma

serum of some of the patients for total base by a method devised by Cullen (4) similar to the Fiske (6) method for the determination of total base in urine. In conjunction with this method the chlorides, carbon dioxide, inorganic phosphates and proteins have also been determined. In this manner the total inorganic anions and cations can be balanced against one another. In table 3 are presented the results obtained on one patient during a period of edema and after diuresis. The edema was very slight. There is no evidence of any change in the level of the total base of the serum in this case and an

TABLE 3  
Case 34295

	Blood sugar	Oxygen capacity	Cell volume	Plasma proteins	Plasma CO <sub>2</sub> content	Plasma NaCl	Serum inorganic phosphate	Total inorganic acid	Total base	Difference base acid	Remarks
	mgm per 100 cc	vols per cent	vols per cent	per cent	vols per cent	grams per liter	grams per liter	mM	mM	mM	
August 23	200	18.0	41.5	6.03*	70.3	5.89	0.035	134.0	154.3	20.4	Slight edema of ankles Weight 147 pounds, in- creasing
August 30	152	16.7		6.45*	68.0	5.90	0.037	133.2	154.9	21.7	No edema Weight 144 pounds, di- minishing pH of plas- ma 7.39

insignificant alteration of the total inorganic acid. If these observations are typical of diabetic edematous conditions it appears that some factor other than the acid-base balance must be active in the production of the edema. It is possible that undernutrition with its attendant loss of body protein may render these subjects more susceptible than normals to the effects of alkali. In this connection one can not but be reminded of the famine edemas that were so prominent in the War (13).

The fact that it is really the acidosis of diabetes and not the ketosis

were receiving adequate diets (See, for examples, nos 10572, 15096 and 22350)

Case 22350, was again admitted to the hospital recently after another attack of vomiting, in a desperate condition. The findings on the second admission are shown in table 4. The same rapid fall in the protein level during the first few days is noticeable. This time, however, the initial protein level is much lower and the subsequent depression proportionately greater. But again, after an interval and with the establishment of proper metabolic conditions the proteins rapidly rise to the normal level. The low proteins and blood concentration in this instance are quite similar to the findings in case 18067, mentioned above. It may be that both are due to the same cause and that the explanation previously advanced for 18067 is unnecessary.

It is needless to point out that without insulin these studies could have led to no more satisfactory conclusions than those of previous observers. The miraculous rapidity with which the clinical and metabolic features of a case can be altered by the use of this drug accelerates the blood changes to such an extent that blood dilution can be studied with less danger from the introduction of complicating factors.

As to the underlying cause of the low proteins in severe diabetes only speculation is as yet permissible. It does not seem absurd to connect them with the general malnutrition and protein wastage that these patients exhibit. Kerr, Hurwitz and Whipple (11) have, to be sure, shown that short periods of starvation do not affect the plasma proteins. It is, however, axiomatic that starvation does not result in nutritional disorders similar to those produced by the prolonged use of unbalanced diets. The same authors (12) have shown that the regeneration of protein after plasma depletion is retarded by inadequate diets. One is tempted to conclude that the low proteins in the plasma in severe diabetes are due to an insufficient production or regeneration which is augmented when the normal nutrition and metabolism are restored.

This again opens the question of the diabetic edemas. Usually in the presence of edema the proteins are low. That this is not invariably the case has been demonstrated. This is to be expected if

volume and reveals the true level of the proteins, which is low. As improvement in the general condition progresses the total amount of protein in the body increases, possibly because of an increased production. In favor of such a theory is the general nature of the con-

TABLE 4  
Case 22350

	Blood sugar	Oxygen capacity	Cell volume	Plasma proteins	Plasma CO <sub>2</sub> content	Remarks
	mgm per 100 cc	vols per cent	vols per cent	per cent	vols per cent	
September 6, 11 p m	462				19.0	At time of admission Patient vomiting continuously. Large amounts of glucose and acetone in urine.
September 7, 9 a m	370			5.06	27.7	Still vomiting continuously, but has received insulin, carbohydrate and fluids. Acetone has diminished.
September 8, 9 a m	233			3.22	47.0	Vomiting has ceased. Improvement continues. Fluid intake larger.
September 10, 10 a.m	257			4.75	28.0	Vomited again this morning, with recurrence of all symptoms.
September 16, 8 a m	270			6.70	59.8	Greatly improved. Receiving adequate diet with large doses of insulin.
September 24, 8 a m	462	16.5	37.6	6.14	57.7	Improvement continues. Weight has increased steadily, without occurrence of edema. pH of plasma 7.48.
October 7, 8 30 a m	130	16.5	40.9	6.98	65.1	Just before discharge from hospital.

ditions associated with low plasma proteins in diabetes. In this series, with the exception of no. 20387, no protein value below 6 per cent was found except in the presence of a severe diabetes with marked evidences of wasting. The same patients invariably had normal plasma proteins when the disease was under control and they

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both low proteins and edema are characteristics of severe diabetes and if edema is provoked by alkalosis, which seldom exists when the blood is dehydrated

#### SUMMARY AND CONCLUSIONS

In mild diabetes unassociated with ketosis or malnutrition the plasma proteins are usually normal

In severe diabetes associated with chronic malnutrition the plasma proteins are usually found reduced. When proper nutrition and metabolic conditions are restored the plasma proteins resume the normal level.

In conditions of severe diabetic toxemia with ketosis the blood becomes concentrated by loss of water. Under these circumstances the plasma proteins appear relatively high. When the toxemia is overcome a rapid dilution of the blood and a reduction of the plasma proteins occur.

The tissue dehydration which is so striking a part of the picture of diabetic toxemias appears to be closely related to the acidosis. Restoration of the alkaline reserve is attended by retention of fluid in the body.

True diabetic edema seldom if ever occurs in the presence of acidosis. A definite alkalosis is, however, not essential to the production of edema. A nutritional factor apparently plays a part in determining the excessive accumulation of fluids, as edemas are characteristic of malnourished patients with severe diabetes who are using insufficient food, and can be eliminated by the administration and utilization of adequate diets. The fact that diuresis can be induced by the production of acidosis indicates that the acid-base equilibrium also plays its part in the etiology of the edema. It is suggested that the state of malnutrition may render the subject peculiarly susceptible to the effects of alkali.

The common association of low plasma proteins with diabetic edema is probably not one of cause and effect but is a result of the fact that both phenomena are characteristic of severe diabetes without acidosis.

## IODINE STUDIES

### I THE AVIDITY OF THE THYROID GLAND FOR VARIOUS IODINE COMPOUNDS IN VITRO

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#### INTRODUCTION

The literature on the relation of iodine to the thyroid gland is extensive, and a comprehensive review of it is not presented in this paper. Excellent reviews exist. The older literature has been reviewed by Horsley (1), the more recent by Wells (2), Marine (3) and Rost (4). Reference will be made only to those observations relevant to the particular problem under consideration.

It is now generally recognized that though the thyroid gland contains little or no iodine in utero (5), after birth it becomes the chief storehouse for this element. In the adult it contains approximately 2 mgm per gram of dry substance (6). The quantity is much less during the first ten years of life and there appears to be no significant variations due to the sex of the individual (7). It is characterized by a marked disproportion between its importance in the human economy and the amount required for its functional purpose. An intake of approximately 500 mgm a year suffices. With the exception of a minute portion which is in inorganic form or in combination with lipoids (8), all exists in organic form. Practically all of it is found in the "colloid" substance (9) of the thyroid gland, the cells containing negligible quantities. All the iodine may be dissolved out from the thyroid in physiological salt solution (10). Since Baumann's discovery, in 1896, that the chief active ingredient of the colloid is an iodine containing compound, very little has been advanced as to the various chemical compositions in which iodine may exist in the gland, with the exception of the identification of thyroxin (11).



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has shown that when the thyroid has little iodine, administration of the latter leads to enormous storage in the gland. The amount may be so great as to produce an acute thyroiditis. Kocher (19) has demonstrated that when iodine is given to a goitrous individual, the concentration of this element in the colloid is increased. Later Marine (2) demonstrated the rate at which iodine may be stored within the gland when fed to animals. Within five minutes after the injection of 50 mgm of potassium iodide into the femoral vein, the concentration may increase several hundred per cent.

Marine (2) first noted that there was a latent period of about 24 hours after iodine administration before a reaction was observed. Boothby and his coworkers (21) later demonstrated such a latent period in the case of thyroxin. The writer (25) in a study of the action of thyroxin, suggested a possible explanation of this phenomenon. Kocher (20) first made the observation that the avidity of the thyroid for iodine may be a function of the concentration of that element already present in the gland. The tissue of an adenomatous thyroid gland does not take up the iodine in the same manner as does the normal thyroid tissue. The importance of this observation appears to have been overlooked, but it has an important bearing on the work to be presented in this paper.

#### OBSERVATIONS

Observations have been made upon the following points

- 1 The effect of exposure of various tissues to a watery solution of iodine, including muscle, serum, spleen, pancreas, kidney and normal thyroid glands

- 2 The effect of exposure of diseased glands to the same solution

- 3 The effect on various tissues and diseased thyroid glands of dilute solutions of

- a Lugol's iodine

- b Acid iodide (made of hydriodic acid and iodine)

- c Potassium iodide

- 4 Effect of treating glands exposed as above with chloroform.

Regarding the solutions used it may be said that when iodine is dissolved in hydriodic acid or in a solution of potassium iodide, there is evidence of chemical combination with the formation of poly-

Great confusion is found regarding the iodine content of the thyroid in disease. It has been frequently found that the iodine content has varied inversely with the degree of epithelial hyperplasia (12) (10) (13). Epithelial hyperplasia is usually regarded as evidence of activity. According to an equal number of observers, thyroid activity was found to vary directly as the iodine content (14). The problem of the relation between the incidence of goiter and the availability of iodine shows still greater confusion. Good clinical results following iodine administration have been observed for many years. Since Baumann's observations that glands in goitrous districts contain less iodine, not only per gram but per gland, the consensus of opinion appears to be that lack of iodine is the causative factor in the production of goiter. In spite of this, however, iodine deficiency cannot be accepted as the only factor. Not only has there frequently been found a normal amount or even an abundance of iodine in goiters, but many well controlled experiments have been reported, which fail to support the view that lack of iodine is the sole cause of goiter (15). Here again clinical experience is corroborative. No satisfactory explanation has yet been offered for success and failure of the same therapy, in two individuals presenting the same clinical picture. Though iodine therapy is successful in the majority of cases of endemic goiter, toxic symptoms have been noted to occur in individuals with apparently the same type of goiter, especially in the case of those living in non-goitrous districts. It has been suggested that iodine may also be useful in toxic goiters, yet it is used as a differential diagnostic test in this condition, because it has been found to intensify the symptoms of toxic goiter (16). Administration of iodine to cases of exophthalmic goiter is known to yield beneficial results. In the majority of instances, however, these are only temporary, and subsequently, by prolonged use, the symptoms may be intensified.

Since the thyroid gland is of such paramount importance in controlling metabolism, and since it appears to require iodine for this function, the selective deposition of this element in the gland is a subject worthy of study. Nagel and Roos (17) first demonstrated that following the administration of iodine to patients, the thyroglobulin becomes rich in iodine, organically bound. Mendel (18)

If the latter procedure is adopted, the filter must be repeatedly washed until all iodides have been washed down. To this solution is then added 10 to 25 cc of chloroform, depending upon the nature of the tissue (normal or pathological). Three cubic centimeters of concentrated sulphuric and 2 cc of concentrated nitric acid are then added, and the flask is rapidly rotated. As the iodine is liberated it dissolves in the chloroform, yielding the characteristic color. The long neck of the flask prevents the loss of material due to spurling during the reaction as the acid is added, and during rotation, and facilitates the collection of the chloroform solution. When all the iodine has been dissolved in the chloroform, the flask is stoppered and inverted. The solution of iodine in chloroform is thus collected and a sufficient quantity is allowed to pass through a dry filter into the colorimetric cup. The intensity of colour is then compared with a solution of chloroform in which the concentration of iodine is known. The usual colorimetric calculations are then applied. Thus

$$\frac{S}{R} \times \frac{I}{X} \times \frac{dR}{dS} \times \frac{G}{I} = \text{milligrams of iodine per gram of tissue}$$

where  $S$  = reading of standard

$R$  = reading of unknown

$X$  = grams of tissue used

$dR$  = dilution of unknown

$dS$  = dilution of standard

$G$  = milligrams of iodine in standard

By this method it is possible to recover the added iodine within 0.2 mgm, an accuracy sufficient for the purpose of this investigation.

## RESULTS AND DISCUSSION

The results of the experiments are given in the accompanying tables. All figures in the tables represent milligrams per gram of dry tissue. In table 1 are recorded the results of the exposure of various tissues of the body to a watery solution of iodine. In table 2 are recorded the results of the exposure of ten diseased glands to the same solution. It will be noted that, *in vitro*, the thyroid gland has a greater affinity for iodine than the other tissues of the body. A striking difference is noted in the avidity in the case of pathological glands, as compared with the normal. In table 3 are recorded the results of the exposure of the same tissues to the different forms of iodine. In table 4 are recorded the results of the exposure of diseased glands to the different forms of iodine. It will be noted that in any particular case in these tables, the amount of iodine recovered differs

iodides (additive combinations)<sup>1</sup> Thus  $\text{HI} + \text{I}_2 \rightleftharpoons \text{HI I}_2$  or  $\text{HI}_3$ . The nature of the polyiodides depends upon whether iodine solution has been saturated with iodide or whether the iodide solution has been saturated with iodine. In the former case the lowest, and in the latter the highest polyiodides, are obtained. Thus, though Lugol's solution, and the acid iodide are made of iodine and iodide, it is obvious that the four solutions used in this investigation differ from each other chemically and physically.

### TECHNIQUE

The tissues were first cut up finely and desiccated in an oven at  $110^\circ\text{C}$  to a constant weight. They were then pulverized in a mortar and passed through a gauze sieve. Any gross particles of fibrous tissue were thus separated. An approximately equal portion (about 1 gram) was then exposed overnight to the various iodine solutions. Each flask contained approximately 500 cc of an 0.020 per cent solution of total iodine. This concentration was chosen for the purpose of uniformity, since it is approximately the maximum concentration possible in the case of a watery solution of crystalline iodine. The following day the supernatant fluid was decanted and the tissue washed repeatedly with distilled water until the wash water was clear. The tissues were then again dried at  $110^\circ\text{C}$  to a constant weight. Each specimen was then divided, approximately, into two equal portions. The iodine content was then determined in a known quantity of one portion. The other portion was kept for chloroform treatment which consisted in the exposure to chloroform overnight. The following day it was washed repeatedly with chloroform, dried to a constant weight and the iodine content determined.

The method of iodine determination was essentially the same as that described by Rouboudin (22), and subsequently employed by Baumann and Roos (23) and Marine and Williams (24), except that colour comparisons were made with the use of a Duboscq colorimeter, and the standard was made by dissolving resublimed iodine in chloroform. In detail it is as follows.

To a known quantity of the tissue (about 0.5 to 1 gram) in a nickel crucible, 10 cc of a 20 per cent solution of KOH are added, and allowed to remain in an oven at  $110^\circ\text{C}$  for about 30 minutes. The uniform mixture is then evaporated slowly to dryness, avoiding any spurting. This is then carbonized. Potassium nitrate is then added to the carbonized mass and the mixture is heated until a clear flux is obtained. The latter is dissolved in hot water, cooled and quantitatively washed into a 500 cc volumetric flask. It is preferable to filter the solution into the flask.

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<sup>1</sup> The results of a comparative study of the clinical use of Lugol's iodine and other polyiodides for exophthalmic goiter in this Hospital, will shortly be published.

adsorbed. This was based upon the observation that, in certain instances, within 5 to 6 hours after exposure of the thyroid tissue to the solution, the latter would become almost colorless. It might be recalled here that weak solutions were used (1 cc = 0.2 mgm I). Another possible explanation was that the iodine united with the

TABLE 3

*Effect of exposure of various tissues and normal thyroid glands to different forms of iodine solutions*

Tissue	Acid iodide	Lugol's solution	Potassium iodide	Watery iodine	Maximum variation
Muscle	0	0.61	Trace	0.46	0.61
Serum	1.79	1.05	Trace	1.61	1.79
Spleen	2.62	Trace	Trace	1.89	
Pancreas	Trace	Trace	Trace	Trace	
Kidney	0.37	0.40	Trace	1.14	1.14
Thyroid (normal)	Trace	Trace	Trace	1.20	1.20
Thyroid (normal)	3.9	3.1	2.8	5.0	2.20
Thyroid (normal)	5.4	5.8	3.0	6.2	3.20

\* Milligram per gram dry weight.

TABLE 4

*Effect of exposure of diseased thyroid glands to various forms of iodine solutions*

Tissue	Acid iodide	Lugol's solution	Potassium iodide	Watery iodine	Maximum variation
S-23-232	36.6*	43.0	49.3	12.1	37.2
S-24-1062	19.7	11.0	7.7	16.0	12.0
S-24-225	71.8	74.8	28.2	21.8	53.0
S-24-194	94.6	57.5	29.6	44.4	65.0
S-23-39	62.4	34.4	25.0	20.9	41.5
S-23-277	27.9	18.9	42.2	27.5	23.3
S-24-999	14.0	5.2	1.8	4.4	12.2
S-23-119	79.2	38.0	26.5	18.5	60.7
L-135	92.1	69.1	59.0	29.7	62.4
Sloan J	35.1	45.1	50.0	51.0	15.9

\* Milligram per gram dry tissue.

lipoids present. The thyroid gland, unlike other glands of internal secretion, has, however, been found to be poor in lipoids (26). However, it appeared reasonable to test these possibilities, by treating the glands, after exposure to iodine, with chloroform, and comparing the iodine content before and after such treatment. If either of these

with the type of iodine solution. In the case of body tissues other than the normal thyroids, the quantities of iodine recovered are very small or negligible. The avidity of the normal thyroid glands for iodine is again noted to be greater than in the case of the other tissues. Table 4 shows strikingly the results in the case of the diseased glands.

TABLE 1  
*Effect of exposure to watery solution of iodine*

Number	Before exposure	After exposure	Tissue
1	0	0.46*	Muscle
2	0	1.61	Serum
3	0	1.89	Spleen
4	0	Trace	Pancreas
4	0	1.14	Kidney
6	0.45	1.20	Normal thyroid
7	Not determined	5.00	Normal thyroid
8	Not determined	6.20	Normal thyroid

\* Milligram per gram dry tissue

TABLE 2  
*Effect of exposure of diseased thyroid glands to a watery solution of iodine*

Number	Before exposure	After exposure	Tissue
1		12.1*	S-23-232
2		16.0	S-24-1062
3	0.14	20.9	S-23-39
4	1.20	21.8	S-24-225
5		55.4	S-24-63
6		44.4	S-24-194
7	0.36	18.5	S-23-119
8	1.10	27.5	S-23-277
9	Trace	29.7	L- 135
10		41.1	S-23-194

\* Milligram per gram dry weight

Here it will be noted that not only is the avidity greater in each case than the normal for any particular solution, but with any one gland differences as much as 60 mgm. per gram may be noted, depending on the type of solution to which the latter was exposed.

Two possible explanations of this phenomenon, other than chemical combination, suggested themselves. One was that the iodine was only

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explanations was correct, a marked diminution in the iodine content would be expected after the treatment with chloroform. In table 5 are recorded the results of ten such observations. The amount of iodine taken up by the different glands varied from 3 to 99 mgm per gram. No significant change was noted in the iodine content in any case after chloroform. Of the little change noted, there was no relation between the loss of iodine after chloroform treatment, and the amount taken up by the gland. It is obvious, therefore, that this phenomenon cannot be explained readily by adsorption or chemical combination with lipoids.

TABLE 5  
*Effect of treatment of exposed glands with chloroform*

Number	Before treatment	After treatment	Difference
1	20.9*	20.4	0.5
2	19.7	18.9	0.8
3	7.7	7.1	0.6
4	16.0	15.9	0.1
5	3.0	2.2	0.8
6	4.2	4.2	0
7	26.0	25.9	0.1
8	55.4	53.6	1.8
9	99.8	99.1	0.7
10	35.2	34.4	0.8

\* Milligram per gram dry weight

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# THE EFFECT OF SOME PATHOLOGICAL CONDITIONS UPON DYSPNEA DURING EXERCISE

## I ARTIFICIAL STENOSIS

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### INTRODUCTION

That dyspnea may occur during exercise and that it is more apt to occur under certain pathological conditions are commonplace observations, yet the physiological mechanisms involved are in part obscure. The effects of exercise have been studied mainly on normal individuals and relatively few observations have been made on patients or on normal persons who have been subjected to abnormal conditions. Pathological conditions may alter the response to exercise in various ways. Thus a given exercise may cause an excessive rise in the metabolic rate, or a given rise in the metabolic rate may be accompanied by an excessive stimulation of the respiratory center, whether through nervous influences, oxygen want, excess carbon dioxide, the formation of non-volatile acids or other factors. Furthermore pathological conditions may reduce the capacity of the external respiratory organs to meet the demands made upon them during exercise. From one or more of these causes, the individual finds it unusually difficult to cope with the respiratory strain imposed by the exercise. The resulting respiratory effort constitutes what we know as dyspnea.

Dyspnea may result when the passage of air through the larger air passages is obstructed, as occurs in stenosis of the larynx and trachea. The effect of such stenoses upon the respiration can be studied experimentally in man by having a normal subject breathe through a tube that has been artificially narrowed to any desired extent.

Using such a method, Morawitz and Siebeck (1) found that the respirations altered immediately after the obstruction had been introduced and before there had been time for any change to occur in the composition of the alveolar air or of the blood. Unless the obstruc-



adjusted that the body weight of the subject sufficed to keep the steps moving at a rate of 80 per minute, as timed by a metronome. The subject (A W H) breathed through a rubber mouth piece, the nose being closed with a clip. By means of flutter valves, the expired air was collected either in the recording spirometer described by Slonaker (3), or in a series of Douglas bags, the latter being used for gas analysis. Artificial stenosis was produced by introducing a bored cork into the rubber mouth piece. Two corks were used for this purpose. The larger bore, 8 mm in diameter and 27 mm long, caused only a slight sensation of obstruction during quiet breathing and moderate discomfort during the exercise. The smaller bore, 6 mm in diameter and 25 mm long, produced some discomfort during rest and progressive embarrassment during the exercise. With this degree of stenosis exercise could be continued only slightly beyond two minutes when air was breathed. Even the ascent of 100 steps, the standard used for recovery curves, caused marked respiratory distress.

Before the exercise a record of the respiratory volume or of the respiratory interchange of gases was obtained with the subject either sitting or standing. No effort was made to obtain basal figures for the position assumed either by a prolonged preliminary rest period or by omitting breakfast. The exercise consisted in ascending the treadmill at a uniform rate of 80 steps per minute. Recovery records were obtained with the subject sitting or standing, after having climbed 100 steps. In other experiments the respiratory changes were studied during the exercise itself. Particular attention was paid to the initial 100 steps and to the later period, when after three or four minutes of continued exercise, the conditions were approximately stationary.

The external work performed per minute consisted in lifting the body weight eighty 7-inch steps. With a body weight of  $157\frac{1}{2}$  pounds, this would be equivalent to 1016 kilogrammeters of work per minute. The minute utilization of oxygen after four or more minutes of exercise approximated 2000 cc. If we subtract a resting oxygen consumption of 315 cc, there was left an excess consumption of 1685 cc per minute which was equivalent to 8 194 calories, if a respiratory quotient of 0.85 be assumed. The heat equivalent of the external

tion was marked enough to cause a feeling of distress, no increase in the alveolar tension of carbon dioxide could be demonstrated, and in no case was the oxygen content of the blood altered. Immediately after introducing the obstruction, the respirations became slower and deeper, the midvolume of the lung became greater, and the dead space was increased. With milder degrees of stenosis, these changes disappeared immediately after the obstruction was removed. The prompt appearance and disappearance of these changes, together with the absence of demonstrable alterations in the composition of the alveolar air indicated that they were caused by changes in the nervous rather than the chemical control of respiration. If the stenosis was more marked and subjective feelings of distress were experienced, an increased carbon dioxide tension in the alveoli was found. Under such circumstances a chemical stimulation of the respiratory center contributed to the production of the altered respiration. Haldane (2), who also studied the effect of artificial stenosis upon the respiratory mechanism, found that the slowing of respiration preceded the increased depth of the individual respirations. He attributed the slowing to nervous influences. In accordance with the Hering-Breuer theory of nervous control, the respirations became slower, because, owing to the stenosis, it took longer for the lungs to reach a given position of in- or expiration. This slowing decreased the minute ventilation, carbon dioxide accumulated and stimulation of the respiratory center deepened the respirations. Thus according to Haldane the slow and deep respirations, characteristic of stenosis, depend in part upon nerve reflexes, in part upon carbon dioxide retention. Haldane found furthermore that very marked obstructions or moderate obstructions, when combined with muscular exercise, led to oxygen want and that when this occurred a more rapid and more shallow type of breathing appeared.

In the present study the effects of stenosis upon the volume of pulmonary ventilation during and after exercise were studied. Changes in the composition of the respired air were also investigated.

#### METHOD OF STUDY

In order to insure a uniform amount of exercise a treadmill was used, the steps being 7 inches high. On this treadmill a brake was so

and a more gradual increase during the succeeding minute or two (table 2, fig 1) Thereafter the volume of respired air remained approximately constant or increased very slowly The rise in volume of ventilation observed at the onset occurred so promptly that it must be attributed in part to nervous influences, the gradual increase during the subsequent two minutes was due to an adjustment to the new metabolic conditions, while the final constant or nearly constant

TABLE 2

*Effect of obstruction upon minute volume of pulmonary ventilation during exercise at 80 steps per minute*

Volumes are expressed in liters and are not corrected for temperature or pressure.

	No Obstruction	8 mm. Obstruction	6 mm. Obstruction
Number of experiments averaged	7	3	3
Resting rates	9.1	9.9	9.1
Minute volume in liters during exercise for successive 10 second periods after the start	20.3	19.6	15.8
	22.4	20.9	16.0
	26.5	22.6	19.4
	29.3	27.9	21.3
	32.2	29.2	24.0
	36.1	33.0	25.7
	39.0	33.4	28.3
	41.0	37.5	28.0
	45.6	38.9	27.5
	47.5	41.5	28.3
	50.8	42.5	28.0
Minute volume after three minutes exercise	52.6	43.7	
Number of experiments averaged	5	4	

The exercise consisted of ascending steps at 80 steps per minute.

minute volume indicated either that a steady state had been established or that the maximum volume of ventilation had been reached At each stage, the effect of obstruction in lessening the volume of respired air was evident In the case of the 8 mm bore, the primary rise was only slightly less than without obstruction but the difference between the two gradually increased The steady state eventually established showed a minute volume of approximately 44 liters, while without obstruction it was 53 liters With the 6 mm bore, the pri-

work performed was 2 380 calories and the net mechanical efficiency was 29.0 per cent<sup>1</sup>

#### EFFECT OF STENOSIS UNDER RESTING CONDITIONS

The 8 mm bore, which produced hardly any sensation of obstruction during rest, did not definitely influence the respiratory rate, the minute volume or the composition of the expired air. The 6 mm bore invariably slowed the respiratory rate (table 1). The average minute volume of air breathed was lessened in one series of experiments (table 5) but uninfluenced in the other (table 4).

TABLE 1

*Effect of stenosis on respiratory rates*

	No obstruction	8 mm bore	6 mm. bore
	<i>per minute</i>	<i>per minute</i>	<i>per minute</i>
Resting	16.5 [10]	17.1 [8]	13.6 [6]
First minute of exercise	27.6 [9]	21.0 [4]	16.7 [5]
Second minute of exercise	28.3 [9]	20.6 [4]	17.7 [4]
Continued exercise	27.8 [5]	20.5 [2]	

The unbracketed figures represent respiratory rates per minute. The bracketed figures give the number of observations averaged.

#### EFFECT OF STENOSIS DURING EXERCISE

Obstruction uniformly slowed the respiratory rates during exercise (table 1). Rates characteristic of the degree of obstruction appeared with the first few steps of exercise and remained practically constant thereafter. Changes in volume of respiration subsequent to the first few seconds of exercise were therefore due almost entirely to changes in the size of the individual respirations. The volume of respiration was invariably reduced by the 6 mm bore and to a lesser extent by the 8 mm bore. In all records the spirometer showed an abrupt increase in respiratory volume during the first ten seconds of exercise.

<sup>1</sup> If the resting oxygen utilization were 270 cc—a level reached by this subject in other experiments after a longer rest period—and if a respiratory quotient for the excess metabolism due to exercise were 1.00, then the heat equivalent of the excess oxygen used would be 8 731 calories and the net mechanical efficiency 27.3 per cent.

more pronounced with the marked obstruction. These obstructions therefore led to a retention of carbon dioxide in the body during the earlier period of exercise. With the 8 mm bore the exercise could be continued until a steady state was attained. At this later stage of

TABLE 4

*Summary of table 3. Respiratory interchange during and after exercise, results given as average minute volume*

- 1 Subject standing at rest.
- 2 During first continuous 100 steps
- 3 During first two minutes rest after 100 steps
- 4 During second two minutes rest after 100 steps
- 5 Minute volume during fourth minute or later of continuous exercise.

	1	2	3	4	5
Minute volume uncorrected for temperature or pressure					
	liters	liters	liters	liters	liters
No obstruction	9.21	33.62	22.45	11.95	53.41
8 mm. bore	9.66	27.46	22.92	10.53	41.90
6 mm. bore	9.58	23.05	22.88	11.91	
6 mm. bore, breathing 36 per cent O		16.86			28.33
CO elimination					
	cc per minute	cc per minute	cc per minute	cc per minute	cc per minute
No obstruction	258	1,047	732	307	1,759
8 mm. bore	274	970	750	269	1,763
6 mm. bore	278	777	836	331	
6 mm. bore, breathing 36 per cent O		685			1,506
O <sub>2</sub> absorption					
	cc per minute	cc per minute	cc per minute	cc per minute	cc per minute
No obstruction	320	1,377	786	331	1,987
8 mm. bore	335	1,340	808	301	2,010
6 mm. bore	339	1,198	969	333	

exercise the carbon dioxide eliminated was equal to that eliminated without obstruction. The restricted volume of ventilation was compensated by a higher percentage of carbon dioxide in the expired air. From this we may infer that the tension of this gas was increased both in the alveoli and in the blood, an inference supported by the retention of carbon dioxide that had occurred during the early stage of exercise.



mary rise in minute volume was distinctly less, and with the second minute of exercise a maximum minute volume of less than 30 liters was attained. This was evidently insufficient for the establishment of a steady state, for increasing distress terminated the exercise at about the end of the second minute.

The effect of restricted pulmonary ventilation upon the respiratory

EFFECT OF STENOSIS UPON PULMONARY VENTILATION  
DURING EXERCISE

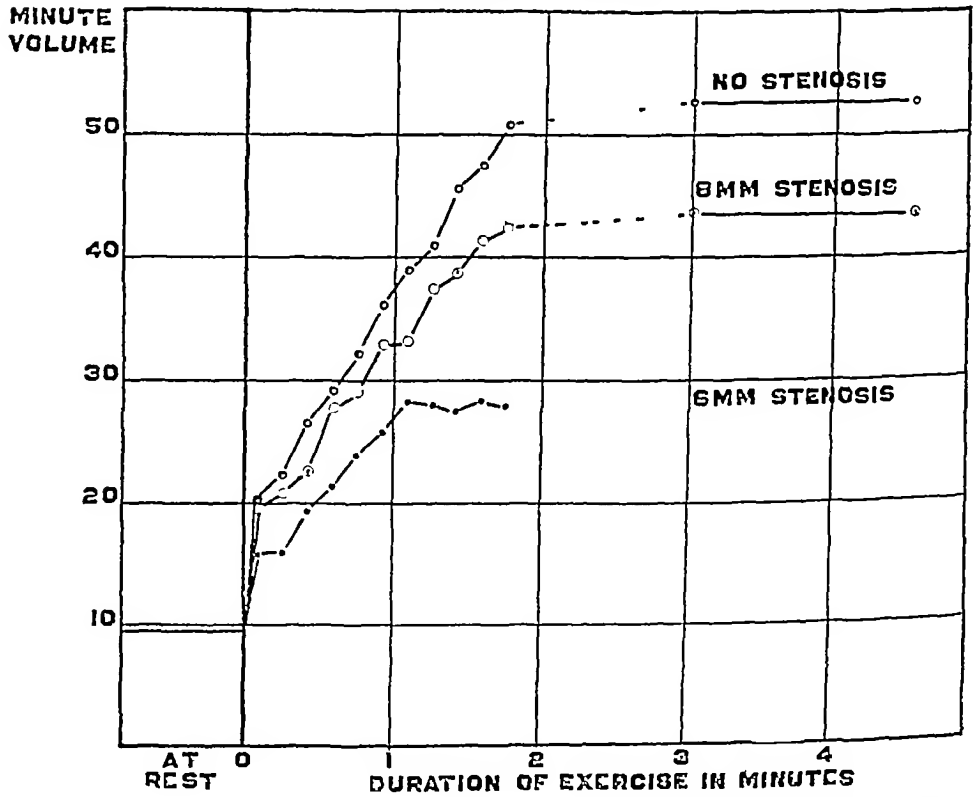


FIG 1 EFFECT OF STENOSIS UPON MINUTE VOLUME OF RESPIRATION DURING EXERCISE

Constructed from table 2

interchange of gases was studied during the first minute and a quarter of exercise (100 steps) and during the steady state attained after several minutes of continued exercise (table 4). During the first minute and a quarter of exercise, the lessened volume of ventilation caused by stenosis was accompanied by a corresponding relative reduction in the amount of carbon dioxide eliminated, this effect being much

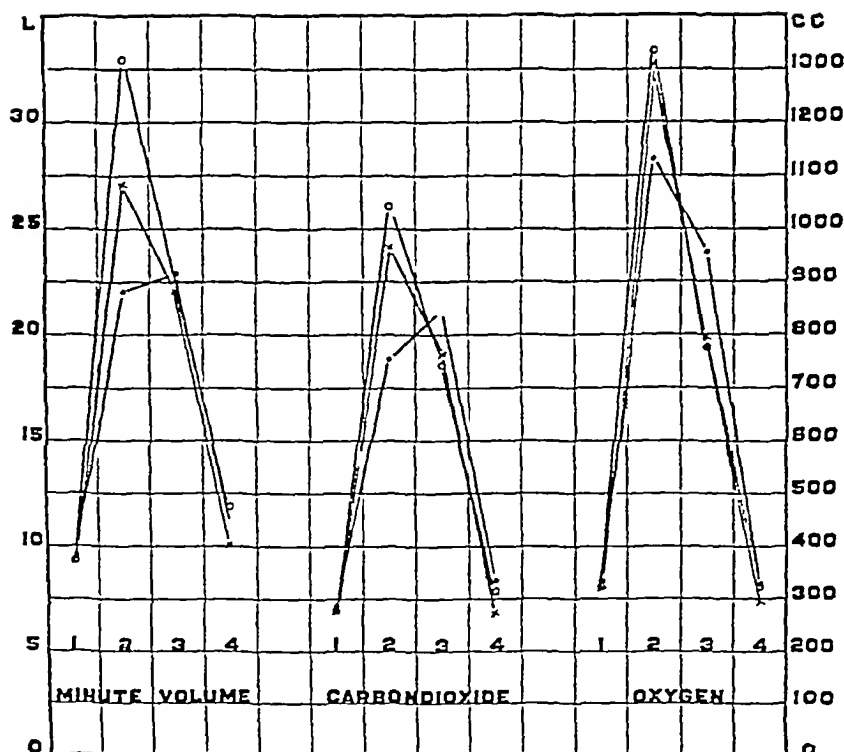


FIG 2 EFFECT OF STENOSIS UPON RESPIRATORY EXCHANGE DURING AND AFTER CLIMBING 100 STEPS

Constructed from table 4 Minute volumes of respiration, carbon dioxide output and oxygen absorption during, (1) rest standing, (2) exercise (100 steps), (3) first two minutes after exercise, and (4) second two minutes after exercise

○ = no obstruction

x = 8 mm obstruction

● = 6 mm obstruction

TABLE 5

Summary of minute volumes of respiration during successive minutes of rest after climbing 100 steps at a rate of 80 steps per minute

	Number of observations averaged	Resting minute volume	Minutes after exercise				
			First	Second	Thrd	Fourth	Fifth
No obstruction	10	8.5	21.9	14.1	10.4	9.5	9.0
8 mm bore	8	8.7	26.3	15.3	10.5	9.6	9.6
6 mm. bore	7	7.7	24.9	15.6	10.6	8.8	8.8

The amount of oxygen absorbed was not appreciably influenced by the 8 mm bore either during the first minute and a quarter of exercise or during the later steady period. To compensate for the lessened volume of ventilation a higher percentage of oxygen was removed from the respired air, but there was no reason to assume that this obstruction caused any oxygen want in the body. The subjective discomfort which led to increased respiratory effort, was caused solely by the increased tension of carbon dioxide.<sup>2</sup>

During the first minute and a quarter of exercise with the 6 mm bore, the amount of oxygen absorbed was definitely less than when no obstruction was used, the difference averaging 179 cc per minute in the two experiments performed (table 4). The rapidly growing distress which terminated the exercise at about the end of the second minute of exercise must therefore be attributed in part to oxygen want and in part to an accumulation of carbon dioxide in the body. When a mixture containing about 36 per cent oxygen was breathed the respiratory distress during exercise with the 6 mm bore was distinctly less and the stair climbing could be continued for about four minutes instead of two. The volume of pulmonary ventilation during the first 100 steps was less than when air was breathed (table 4), presumably because with less distress less effort was made to ventilate the lungs. During this period less carbon dioxide was eliminated. Even in the fourth minute of exercise when the percentage of carbon dioxide in the expired air had risen markedly, the total elimination was distinctly short of that put out during continuous exercise with unobstructed breathing. Under these conditions, *i e*, breathing an oxygen-rich mixture through the 6 mm bore, an accumulation of carbon dioxide in the body was the probable cause of the final termination of exercise.

#### EFFECT OF STENOSIS UPON RECOVERY FROM EXERCISE

Recovery records were obtained for the four or five minutes following taking 100 steps. These showed in every instance that the main portion of recovery was very rapid (fig 2). The final stage of recovery

<sup>2</sup> In unpublished experiments by Barnett, Lewis and Hewlett no increase of lactic acid in the urine was found, even after two minutes exercise with the more marked obstruction.

tilation was insufficient for the establishment of a steady state With the larger bore a steady state could be established with a minute volume of approximately 44 liters, in contrast with the minute volume of approximately 53 liters when breathing was not obstructed

4 When breathing was obstructed, more carbon dioxide was retained during the early stage of the exercise With the 8 mm bore, when a steady state was later established, the minute output of carbon dioxide was the same as with unobstructed breathing owing to the higher concentration of this gas in the expired air

5 With the 8 mm bore oxygen absorption was almost unaffected With the 6 mm bore oxygen absorption during the first minute and a quarter was definitely lessened and lack of oxygen contributed to the early discontinuance of the exercise If a mixture rich in oxygen were breathed, the early distress was less marked and exercise could be continued longer Eventually however it was discontinued, presumably because, despite a high concentration of carbon dioxide in the expired air, the necessary amount of this gas was not eliminated

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Measurement of Dyspnea

ery could not be accurately studied in our experiments because the resting periods which might have served as a basis for comparison had not been obtained after a sufficient preliminary rest period. After this short and moderate exercise, recovery was almost completed during two minutes of rest. At the end of this time the oxygen consumption had returned to the previous resting level, the carbon dioxide output had approached or equalled this level and only the ventilation remained somewhat greater than before the exercise began. During the first two minutes of recovery the excess carbon dioxide retained and in the case of the 6 mm bore the excess oxygen wanted developed as a result of obstruction had already been practically equalized. The respiratory volumes during the first two minutes of recovery were slightly greater as a result of the obstructions but the differences were not large, that for the 8 mm bore being if anything slightly greater than that for the 6 mm bore. These recovery curves of ventilation gave no indication of the distress experienced during exercise. This point is of interest mainly because in certain other forms of exercise dyspnea the volume of ventilation during recovery is materially greater than when the exercise is accomplished without respiratory effort. Hunt and Dufton (4) have indeed proposed a numerical measure of dyspnea based on the increase of pulmonary ventilation during recovery from measured exercise. It is evident however that such a measure could not be applied to the dyspnea provoked by obstruction.

#### SUMMARY AND CONCLUSIONS

- 1 Artificial obstruction to respiration was produced by breathing through bored corks, the bores being 8 mm and 6 mm in diameter.

- 2 Under resting conditions the 8 mm bore produced but little subjective and no demonstrable objective effects. The 6 mm bore caused slight subjective discomfort, and invariably slowed the respiratory rate. In one series of experiments it lessened the average minute volume of respired air.

- 3 During exercise both bores reduced the respiratory rate and lessened the minute volume of respired air. With the smaller bore a maximum minute volume of approximately 30 liters was attained, but the rapidly increasing distress indicated that this amount of ven-

TABLE 3—*Continued**Oxygen absorption*

No obstruction

Date	1		2		3		4		5	
	cc per minute	per cent.	cc	per cent	cc	per cent	cc	per cent	cc.	per cent
4/ 1/24	322	3 80	1,732	4 58	1,617	4 06	627	2 86		
4/ 9/24	338	4 00	1,712	4 47	1,526	3 68	698	3 27		
4/14/24	305	3 86							2,048	4 04
4/15/24	315	3 72							1,927	4 03

8 mm obstruction

4/ 3/24	360	4 05	1,709	5 59	1,599	3 89	640	3 25		
4/18/24	330	3 56							2,020	5 24
4/21/24	331	3 73							1,999	5 27
4/22/24	319	3 91	1,642	5 16	1,631	3 86	565	3 04		

6 mm. obstruction

4/ 4/24	348	3 89	1,401	5 36	2,081	4 98	714	3 30		
4/23/24	330	3 88	1,596	5 84	1,795	4 31	617	2 83		

TABLE 3

*Effect of stenosis on respiratory exchange during and after exercise*

- Period 1 Minute volume, subject standing at rest  
 Period 2 During first 1½ minutes of exercise (100 steps)  
 Period 3 During first two minutes of rest after climbing 100 steps  
 Period 4 During second two minutes of rest after climbing 100 steps  
 Period 5 Minute volume during fourth minute or later of continuous exercise

*Volume of ventilation uncorrected for temperature or pressure*

No obstruction

Date	1	2	3	4	5
	<i>liters per minute</i>	<i>liters</i>	<i>liters</i>	<i>liters</i>	<i>liters</i>
4/ 1/24	9 42	42 05	44 31	24 39	
4/ 9/24	9 27	42 00	45 48	23 42	
4/14/24	8 99				55 02
4/15/24	9 18				51 89

8 mm obstruction

4/ 3/24	9 80	33 7	45 3	21 7	
4/18/24	10 08				41 95
4/21/24	9 78				41 86
4/22/24	8 97	34 96	46 4	20 42	

6 mm obstruction

4/ 4/24	9 77	27 5	45 6	23 6	
4/23/24	9 39	30 12	45 92	24 05	

*Carbon dioxide elimination*

No obstruction

Date	1		2		3		4		5	
	<i>cc per minute</i>	<i>per cent</i>	<i>cc</i>	<i>per cent</i>	<i>cc</i>	<i>per cent</i>	<i>cc</i>	<i>per cent</i>	<i>cc.</i>	<i>per cent</i>
4/ 1/24	265	3 13	1,300	3 44	1,486	3 73	651	2 97		
4/ 9/24	273	3 23	1,318	3 44	1,440	3 48	577	2 70		
4/14/24	238	2 87							1,840	3 63
4/15/24	255	3 02							1,678	3 51

8 mm obstruction

4/ 3/24	287	3 23	1,202	3 93	1,488	3 62	561	2 85		
4/18/24	277	2 99							1,785	4 63
4/21/24	273	3 08							1,741	4 59
4/22/24	258	3 16	1,222	3 84	1,512	3 58	515	2 77		

6 mm obstruction

4/ 4/24	286	3 19	887	3 52	1,722	4 12	694	3 21		
4/23/24	270	3 17	1,055	3 86	1,620	3 89	631	2 89		

# THE MECHANISM OF DEATH FROM QUINIDINE AND A METHOD OF RESUSCITATION, AN EXPERIMENTAL STUDY

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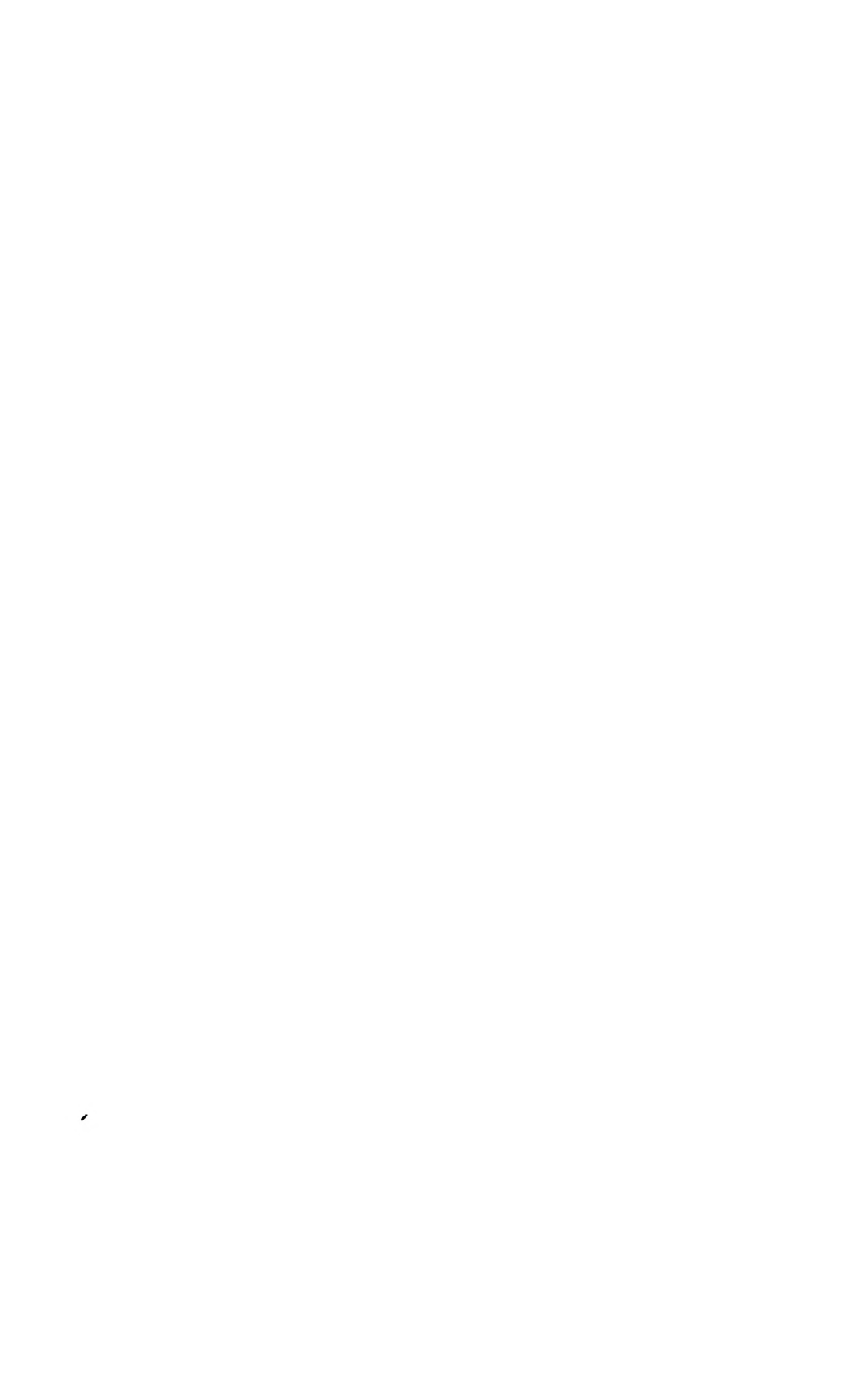
## INTRODUCTION

Fatalities have occurred not infrequently during the clinical administration of quinidine in patients suffering from heart disease. At first particular emphasis was attached to the rôle that embolic phenomena played in producing unexpected deaths (1, 2, 3). Later it was found that during quinidine administration occasionally there developed increased irritability of the ventricles, as evidenced by the appearance of extraventricular systoles and ventricular tachycardia (4, 5). It was thought, therefore, that ventricular fibrillation, the most extreme type of ventricular irritability, might explain some sudden deaths following quinidine.

It soon became apparent that neither of these explanations could account for a further group of such fatalities. This latter feature was particularly impressed upon us in our clinical experience for, in the three fatalities that occurred in the wards of the Peter Bent Brigham Hospital following quinidine therapy, post-mortem examination failed to show any evidence of emboli, and in one of the cases death was not instantaneous. In this patient, a peculiar toxic state resulted, somewhat resembling shock, or at least giving the appearance of unusual respiratory distress for some hours before the fatal termination. We found that similar cases have been reported by others (6, 7), in which this peculiar toxic state occurred. Von Frey (8) in 1918 reported two patients who received 0.2 gram of quinidine five

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## EXPERIMENTAL TECHNIQUE

Sixty adult male cats were used in these experiments. They were etherized, placed on an animal board, and held in the dorsal position. The femoral artery and vein were exposed and 0.1 gram of heparin (15) dissolved in 2 cc. of physiological salt solution was injected intravenously. A cannula was inserted in the left femoral artery and was connected with a manometer apparatus by means of a short piece of rubber tubing. The tubing contained 30 cc. of a 3 per cent acacia solution and 10 mgm of heparin under slight pressure. Stopcocks which separated the manometer and the cannula from the blood stream of the animal were then opened and a graphic record of the arterial pressure was traced on a smoked drum. A pneumograph was placed around the animal's chest and connected with a tambour apparatus, a marking signal and timing device were also in contact with the kymograph. When intratracheal artificial respiration was to be given, the trachea was exposed and opened, by means of a V-shaped incision. A T-shaped cannula was then inserted into the trachea and connected with a compressed air apparatus. The amount of air was regulated by opening and closing one end of the cannula at a rate to correspond with normal breathing. In taking the electrocardiograms, the hair on the two front legs and the left hind leg was clipped and contacts were made by means of gauze strips soaked in salt solution. They were connected with the apparatus in the usual manner. A 5 per cent solution of quinidine bisulphate (manufactured by Howards and Sons, Ilford, England) was freshly made for each experiment. The various doses of quinidine were injected with a tuberculin syringe into the right femoral vein. For the Roentgenograms, the same animal board and technique as employed in a previous study (16) were used, except that in this experiment the current was of 30 milliamperes and the exposure for 2 seconds. It consisted of making rapid exposures of the heart without disturbing the position of the animal. In determining changes in heart size, tracings on smooth white paper were made from the heart shadow, silhouettes were then cut out and weighed in milligrams.

## LETHAL EXPERIMENTS

A series of lethal experiments was carried on in animals of known weight. They indicated, first of all, that there was some relation between the weight of the animal and the size of the dose. We tried to determine whether there was any relation between the speed of administration and the minimal lethal dose, as Cohn and Levy (17) have pointed out that the "greater the fractionation of the dosage, the greater was the amount of drug necessary to cause death." We, likewise, found that the animal could tolerate a much greater amount of the drug when it was divided over a period of time extending from

times a day Three hours after the last dose he found that they presented a picture of cerebral paralysis The patients had a sense of dizziness and increasing warmth, after which they suddenly fainted They grew pale and the respirations became slower and stopped After a short time the pulse could not be felt The patients recovered after artificial respiration was given, and the recovery was accompanied by a stronger heart action He gave epinephrin solution and thought it had a favorable effect He also used caffeine and camphor, but without result As one patient was recovering from the fainting attack following the period of apnea, he noticed some evidence of motor excitability, with hallucinations and grimaces of the face In the second case, while the patient remained unconscious for several hours, on three occasions her condition became worse, this being characterized at first by a standstill of the respiration Von Frey concluded in these cases that there was no evidence of a paralyzing effect of quinidine on the heart muscle, but rather that the results pointed to a central action, the effect being at first on the respiratory center Cordier (9) in 1923 reported his observations on a case of quinidine poisoning This patient, a woman of fifty, suffering from heart disease, was receiving by mouth 0.2 gram of powdered quinidine a day She became indisposed and he noticed that she was breathing with difficulty, developed apnea and finally fainted This occurred three times in the same night He observed no changes in the pulse except a slight acceleration and weakness at the end of the crisis Long periods of apnea were noted by Neuhof (10), and Reid (11) found evidences of respiratory failure following the administration of quinidine in human cases Wiechman (12) observed that in cases of quinidine poisoning there was clinical evidence of damage to the respiratory center This, however, was accompanied by marked changes in the circulation Vasquez and Leconte (13) and Korns (14) did not believe that quinidine ever really caused death Exitus, they thought, was due to some underlying heart condition This brief review indicates that there is considerable confusion as to whether the heart or the respiratory system is the one that becomes intoxicated primarily It was with the purpose of studying the mechanism of death and thereby obtaining information as to whether we had any means of preventing such catastrophes that the following investigation was undertaken

## OBSERVATIONS ON THE BLOOD PRESSURE

As Cohn and Levy (17) and other workers have observed there was a constant sudden drop of the blood pressure following the first injection of quinidine. This was not always related to the size of the dose. We found that the degree of this fall varied between forty and eighty millimeters of mercury or more. There occurred quickly a gradual although incomplete return to the normal level. There was a tendency of the blood pressure to remain low for a longer time when the dose was large, the return also being less complete. The average time between the first drop in the pressure and the return to the next highest level was from five to ten minutes. The drops following successive injections grew less in extent than those following the previous ones, and the recovery of the pressure was also less marked, so that in studying a long chart there was a stairlike effect on the pressure curve, which remained for some time at a level of about 30 to 40 mm of mercury if no further injections were given (fig 1).

The question arose as to whether the fall in the pressure was due to an action on the heart muscle itself or whether it was due to a peripheral vascular dilatation. With this in mind, experiments were performed in which the abdominal aorta was compressed. Referring to figure 2 it will be seen that after obtaining a record of the normal pressure, compression of the abdominal aorta elevated the carotid arterial pressure 75 mm of mercury above the normal level. While the aortic pressure was being maintained an intravenous injection of quinidine produced a fall of about 40 mm, which level was nevertheless 25 mm higher than the normal readings before compression of the aorta. When the abdominal aorta was released there was an immediate fall in pressure to a level that would have corresponded to that obtained from the quinidine if no aortic compression had been employed. Several minutes later when the blood pressure had returned to its highest level, the aorta was again compressed with a similar rise of about 80 mm, bringing the reading to 155 mm, nearly as high as the level reached after the first injection. From this point a second injection of quinidine again produced a fall of about 30 mm, and when the aorta was released a drop occurred which corresponded to about

half an hour to two or more hours than if larger individual doses were given more rapidly. As a result of numerous experiments it was found that 25 to 30 mgm per kilogram were fatal when given in one dose. The total minimal lethal dose, however, if 15 mgm were given every six minutes, was 45 mgm per kilogram. It was possible by giving still smaller divided doses over a period of two hours to administer 100 mgm per kilogram before the lethal effect was obtained. This total dose was four times as great as the minimal lethal amount when one single dose was given. Although the above figures hold in a general way, there were appreciable variations in some experiments. A striking thing about these animals was that practically no ether was required after the first injection, as the quinidine seemed to be sufficient to produce a state of narcosis.

#### NON-LETHAL EXPERIMENTS

In this series of experiments a small single dose was given in order to determine the margin of safety for administration. A single dose of 20 mgm per kilogram caused no appreciable change in the respiration except occasionally a slight slowing. On the other hand, when a dose of 25 mgm per kilogram was given, which was in the vicinity of the lethal dose, there was frequently a brief cessation of the respiration, followed by a slow rate and a gradual return to normal. In about one half of the instances the dose was fatal. In other experiments a total of 76 mgm per kilogram administered in divided doses over a period of two hours was not fatal, but caused some slowing of the respiration and a moderate narcosis.

These results stand out in marked contrast to those obtained when digitalis is given, for here the speed of administration does not alter the minimal lethal dose (18). Some of the animals were returned to the cages, where they completely recovered. The others were used for different purposes. In several it was found that, following injections of quinidine, apparent recovery of the animal took place within a few hours and complete recovery in twenty-four hours, in the sense that similar repeated injections could be given without any evidence of an accumulative action of the previous administration. This corresponds quite well to the observations made by Lewis (19) who found that quinidine was completely excreted in approximately one day.

the previous low level. This experiment is not critical in differentiating the effect of quinidine on the vessels from the direct action on the heart muscle. But the fact that quinidine produced only a partial fall of pressure while the abdominal aorta was completely compressed indicates that the action of the drug, at least in a great measure, is on the abdominal or peripheral vessels. Definite evidence for an additional effect on the heart muscle was obtained from a different angle

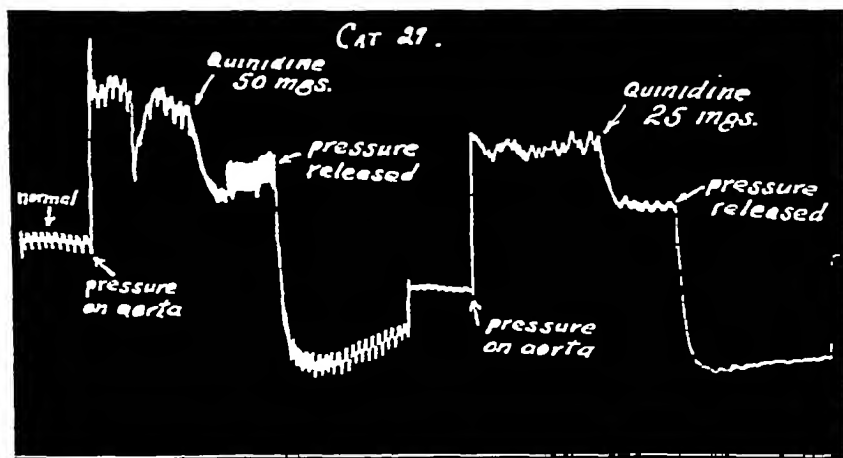


FIG 2 CAT 29 WEIGHT, 3.9 KG. BLOOD PRESSURE RECORD

Note increase in pressure following compression of the abdominal aorta and partial fall as a result of quinidine, with further fall after release of aortic compression

in that the changes seen in the electrocardiograms (see below) were of a type that is found only when the heart muscle is undergoing serious damage

#### OBSERVATIONS ON THE RESPIRATION

Von Frey and Haegeman (20), working on rabbits observed that quinidine had at first a paralyzing effect on the heart muscle and only later affected the respirations. On the other hand, in this investigation we found that a single dose of 30 mgm per kilogram or more caused the respiration to become irregular, then it grew slow and

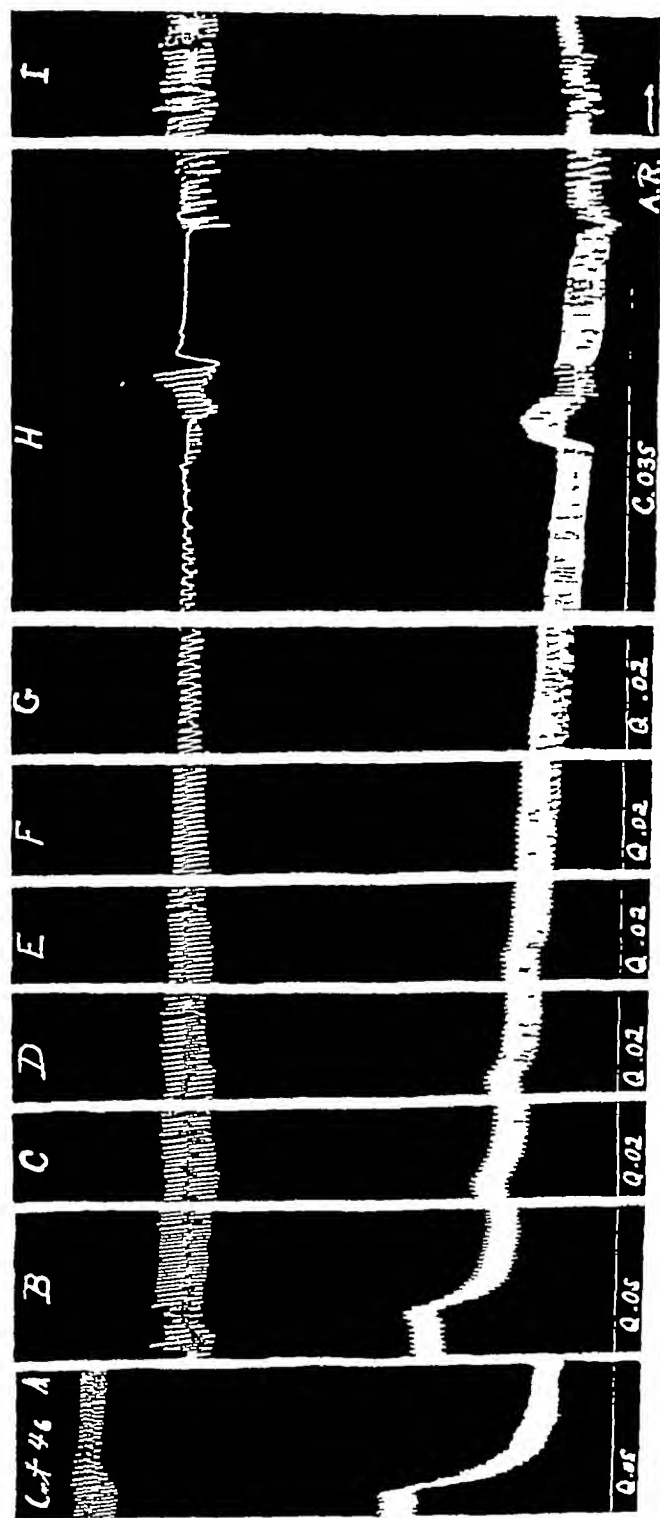


FIG 1 CAT 46 WEIGHT, 4.4 KG. UPPER TRACING INDICATES RESPIRATIONS, LOWER, BLOOD PRESSURE

Strips A to I are portions of the entire experiment. Quinidine doses every twelve minutes indicated below. Note stair-like fall in pressure and gradual cessation of respiration. Caffeine (35 mgm.) produced increase in pressure and transient stimulation of respiration followed by complete arrest. Artificial respiration (A.R.) revived animal, i.e., I shows normal breathing.

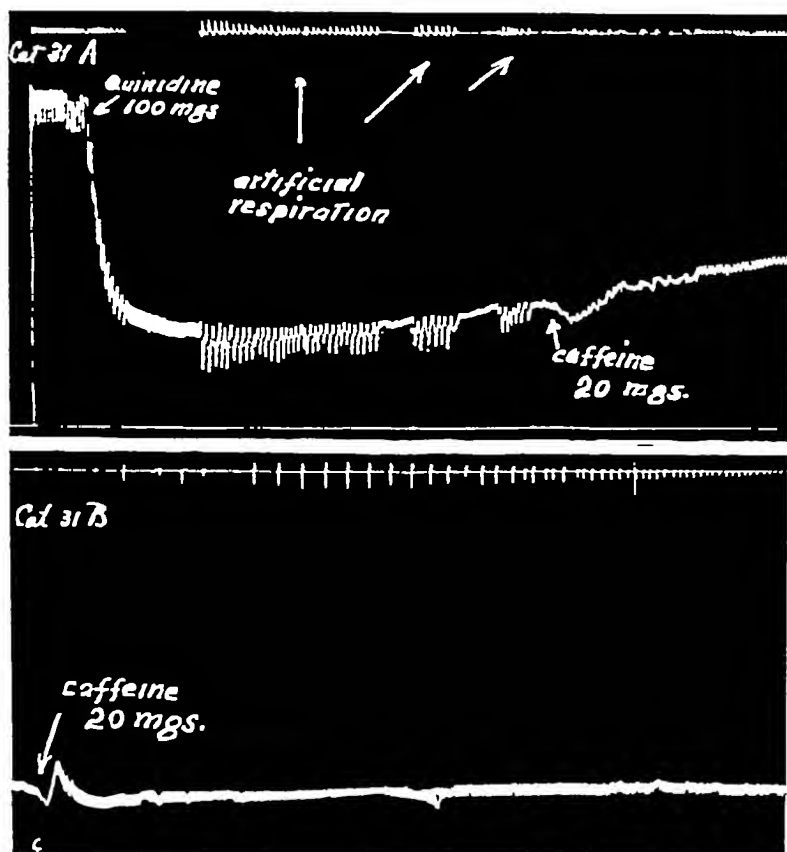


FIG 3A CAT 31 WEIGHT, 5 KGM UPPER TRACING INDICATES RESPIRATIONS, LOWER BLOOD PRESSURE

Note sudden marked drop in pressure following quinidine with cessation of respiration Three periods of artificial respiration failed to institute spontaneous breathing After caffeine was given normal breathing returned

FIG 3B BETWEEN A AND B SUFFICIENT QUINIDINE WAS GIVEN TO PRODUCE CESSATION OF BREATHING

Note that caffeine alone produced return of normal respiration although blood pressure remained low



been given, it was not uncommon for the cat to develop short periods of convulsions. At this stage frequently there was also a livid appearance of the lips and from time to time the cat tossed his head from side to side and threw his legs about limply. In some cats there was a relaxation of the sphincters. The asthenic appearance of the animals closely resembled the appearance of intoxication that one of our patients who died following the oral administration of small doses of quinidine presented during the last few hours of life.

#### MEANS OF RESUSCITATING THE ANIMAL

At the beginning of this study it was our impression that the cats were dying a cardiac or circulatory death. In some experiments heart drugs such as ouabaine, strophanthin, and digitalis were given intravenously when symptoms of catastrophe appeared. We also used the drugs before starting the injection of quinidine with an idea of preventing the deleterious effect on the heart and the circulation. The harmful effects of quinidine were neither prevented nor removed by the use of these drugs. Von Frey and Haegeman (20) made the same observations. Suspecting that the failure might be due to vasodilatation, causing a low blood pressure so that the vital centers failed to receive a sufficient blood supply, we placed the animal head down, but observed no changes in the respirations (fig 4). It quickly occurred to us that the respiratory mechanism was affected, at least in a measure, independently of the circulation. Although the lower state of blood pressure could account for a part of the respiratory distress, it seemed unlikely that it played an important rôle, for it has been observed frequently in these experiments that cats were breathing normally with extremely low blood pressures (fig 1, Section E). As it was felt that caffeine has a stimulating effect on the respiration (21), we thought that it might be of value if the phenomenon were respiratory collapse. Thus in a group of animals we gave a moderately large dose of quinidine, a dose sufficient to produce respiratory embarrassment. At the point when the cat was breathing poorly, and just about the time a standstill in the respiration seemed to be imminent, an injection of caffeine sodium benzoate was given (about 5 mgm per kilogram). In most instances the normal breathing returned (fig 4). Other cats received sufficiently large doses

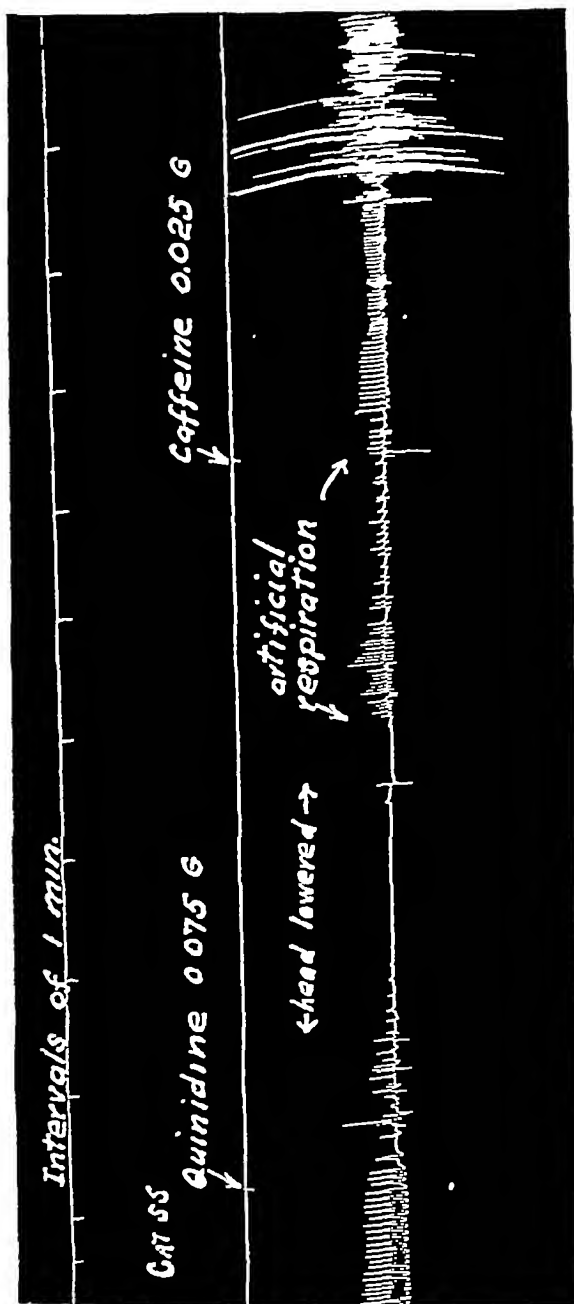


FIG. 1. CAT 55. WIGHT, 2.3 KCM. TOWER LACINE SHOWS RESPIRATIONS

Note cessation of breathing for about two minutes following quinidine, despite lowering of head. Artificial respiration and caffeine caused return of normal respirations. Large swings at end of tracing are due to vigorous movements of animal.

of quinidine to produce complete respiratory failure. One minute after breathing had stopped, an intravenous injection of caffeine was made. In about one half of this series after a few slow and deep breaths, the normal breathing returned (fig. 4).

To determine whether it was possible to prevent untoward symptoms following the administration of dangerous doses of quinidine, the following experiment was done. The animal was given as a control 25 mgm of quinidine per kilogram. The respiration was very definitely affected. The rate was slow and the breathing shallow, but the animal recovered. The next day caffeine was given first and followed by the same dose of quinidine, without noticeable change in the respiration. One day later the same animal was given 30 mgm of quinidine and the respiration stopped, caffeine (5 mgm per kilogram) was given intravenously. The respiratory rate returned to normal and the animal recovered. These experiments were repeated on the same cat and similar results were obtained. The above experiments indicate a definite beneficial effect of caffeine on the untoward depression of the respiratory mechanism following quinidine.

The possibility of saving all cats, either from a large single lethal dose of quinidine or from repeated small doses of such amounts as were usually lethal, was then considered. When the cats stopped breathing, artificial respiration by means of chest massage was given. This procedure failed completely if vigorous or rapid manipulations were used in carrying out the massage. On the contrary, it was quite dependable in cats not hopelessly intoxicated by quinidine when the respiratory movements were made in a slow careful manner to correspond to the normal rate of the respiration. It was felt, however, that with more complete ventilation of the lungs, it might be possible to save the animals more readily. Therefore, intratracheal artificial respiration as described above was undertaken. This method seemed to be far more efficient. Cats given fatal amounts of quinidine either in a single massive dose or in small repeated doses which were sufficient to produce cessation of the respiration for as long as two minutes, were saved by this procedure. Some cats in which automatic breathing had stopped were given artificial respiration for from fifteen to twenty minutes before normal

breathing returned. The heart action during this period of respiratory failure was satisfactory, the blood pressure ranging around 40 mm. One cat which received a very large dose, i.e. 50 per cent larger than the lethal dose, stopped breathing for two minutes. The animal was inverted with no effect. Artificial respiration by the intratracheal method was then instituted and was stopped at intervals, about every minute, to determine if the animal were breathing spontaneously. After nine minutes normal respiration returned. The next day the animal was in good condition and the same dose was repeated. When respiration ceased the cat was again inverted, without any beneficial result. Then artificial respiration was started, but at the same time an intravenous injection of 25 mgm. of caffeine sodium benzoate was given. At the end of one minute artificial respiration was stopped, and at that time the cat was breathing normally (fig 4). Whereas on the previous day it required nine minutes of artificial respiration before the normal breathing returned, in this experiment the cat was breathing after one minute. It seems, therefore, that the combined action of the two procedures, i.e., artificial respiration and caffeine, restored the animal more quickly and more efficiently than artificial respiration alone. In numerous experiments similar recovery of the animals after lethal injections of quinidine occurred.

#### OBSERVATIONS ON THE HEART

Santesson (22) as early as in 1893 concluded that the action of quinidine was essentially a muscle poisoning. Waddell and Cohen (23) in an experimental study on the amphibian heart thought that the quinidine produced its effect through an action on the musculature of the heart and of the circulatory system, and they considered it to be a physiological depressant.

Throughout our experiments, numerous electrocardiograms were taken. In general it was found that with small doses of quinidine transient changes in the ventricular complexes occurred. The duration of the Q-R-S complex was prolonged and the amplitude of the R and S waves was also increased. Frequently there was a slight delay in the P-R interval and even heart block as were noted by Korns (14) and Lewis et al (24). These changes disappeared before the next injection. The general type of curves is similar to those

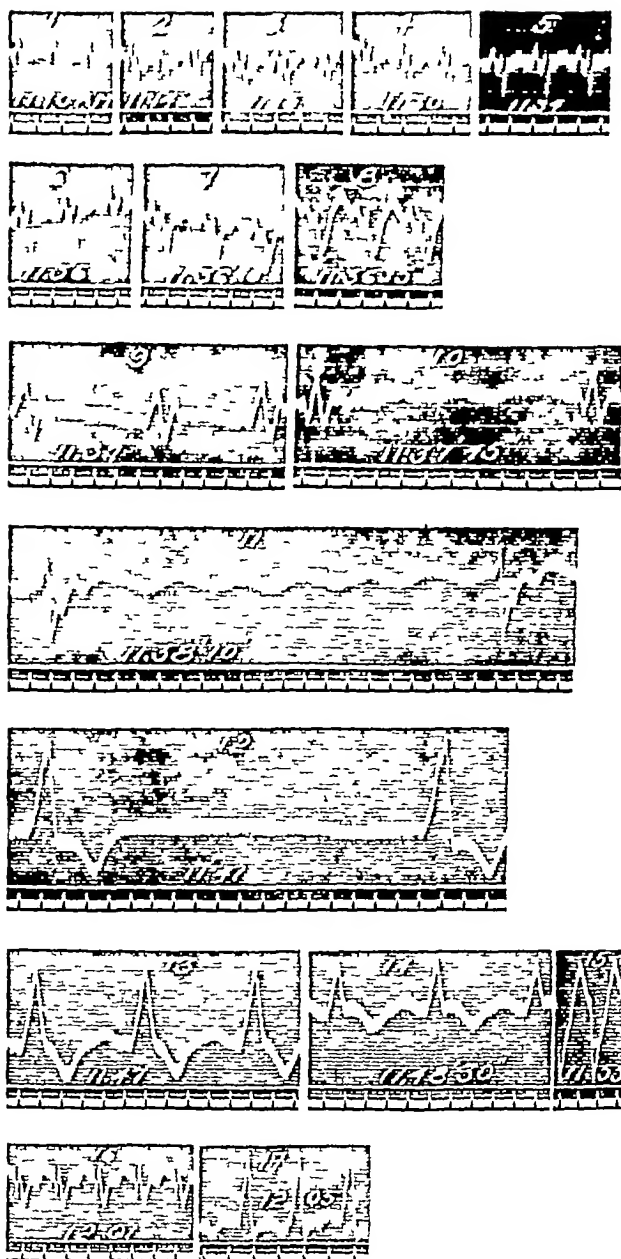


FIG 5 CAT 41 WEIGHT, 3.4 KG. ALL ELECTROCARDIOGRAMS ARE LEAD 2

Timer below measure  $\frac{1}{2}$  second. The time the various tracings were taken is indicated below each curve. No. 1 is normal control. Quinidine 25 mgm given at 11:13 a.m. Quinidine 25 mgm given at 11:29, 15 mgm at 11:33, 25 mgm at 11:35. Respirations stopped at 11:37. At 11:38 caffeine sodium benzoate 20 mgm given. Artificial respiration from 11:40 to 11:50 caffeine 10 mgm at 11:55. Note slight slowing and changes in the complexes with early doses of quinidine and more marked changes with later doses. Striking recovery followed artificial respiration and caffeine.

seen in the upper two sections of figure 5. The electrocardiographic changes became progressively more marked with repeated injections, with a gradually diminishing degree of recovery. The heart rate in most instances was slow and death occurred without fibrillation of the ventricle in the great majority of instances. The final tracings were apt to show bizarre ventricular curves. In those instances where the respirations had ceased entirely and it seemed that the animal would certainly die, electrocardiograms could still be obtained, although they indicated a most grave state of intoxication. Despite the dangerous outlook, artificial respiration was successful, both in reviving the respiratory mechanism and in allowing the heart to be restored so that in about two hours electrocardiograms were essentially normal. An example of recovery after a lethal dose of quinidine was given is shown in figure 5. When such extraordinary curves were obtained and means of resuscitation were not employed, the animal invariably died, but here satisfactory heart as well as respiratory recovery occurred by means of artificial respiration.

By means of roentgenographic examination it was found that within a few seconds following the injection of 22 mgm of quinidine per kilogram there began a diminution in the size of the heart. There was further contraction during a period of seventy seconds and thereafter the heart rapidly dilated to a size greater than the normal control, with eventual return to normal (fig 6). With the subsequent injections of smaller doses of quinidine there was a diminution in the heart size which was not followed by the period of dilation before the gradual return to normal that occurred in the former case. In figure 6 the first changes in the size of the heart as indicated by a decrease in the weight of the silhouettes, although small, are in fact significant. It was generally considered that changes under 10 per cent were within the limit of error (25) and the fall from 0.061 to 0.056 might be within this margin. But the decrease in the heart size continued, as was indicated by the figure 0.051 which could not be considered an error in technique. Furthermore, similar alterations were observed in the same animal, which makes it more certain that a true contraction occurred directly after each injection. There can be no doubt that a subsequent dilatation occurred, for the change from 0.051 to 0.077 is an increase of 50 per cent. Figure 7 likewise shows these changes in the actual roentgenograms.

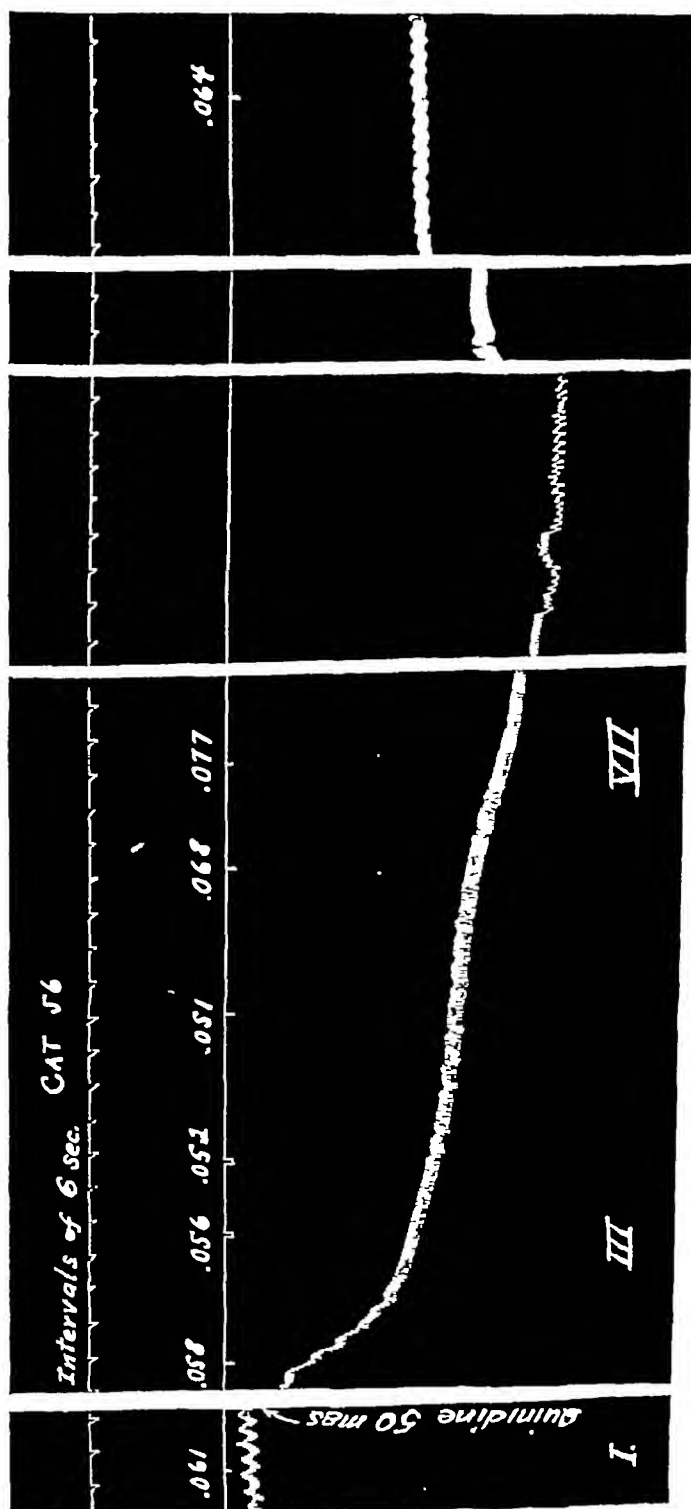


FIG 6 CAT 56 WEIGHT, 2.3 KG. NUMERALS SHOW WEIGHT OF X-RAY SILHOUETTES TAKEN AT MOMENT INDICATED BY SIGNAL JUST BELOW

Note slight decrease in size of heart with the fall in pressure after quinidine, this was followed by a dilatation, as indicated by the figure 0.077 with a final return to normal size (0.064)

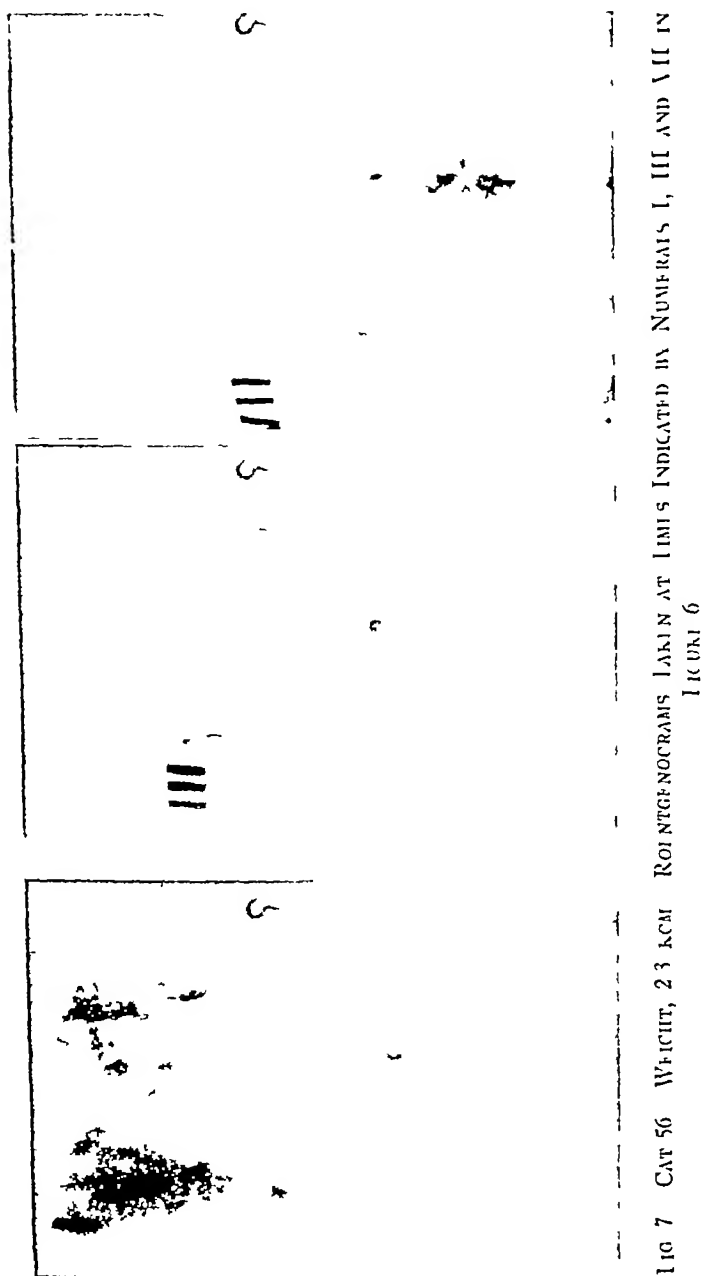


FIG 7 CAT 56 WEIGHT, 2.3 KGM. RADIographs TAKEN AT LUMBAR L5 INDICATED BY NUMBERS I, III AND VII IN  
FIGURE 6

I shows normal control, III shows contraction, and VII shows dilatation



These observations in general are in accord with those of Jackson, Friedlander and Lawrence (26), despite the fact that in their experiments the chest was opened and considerable manipulation of the heart was necessary, whereas here, intact animals were employed. It seems most likely that the initial contraction was related to the sudden fall in pressure and was independent of any local action of the drug on the heart. It was recently shown (16) that with the fall in pressure that accompanied amyl nitrite administration and bleeding there was a similar contraction in the heart. The dilatation that occurred following the initial contraction after quinidine injection probably resulted from direct action of the drug on the heart muscle, as it was about that time that repeated electrocardiograms on the animals showed changes in the ventricular complexes which were indicative of intoxication of the muscle. Six minutes after quinidine administration, the heart size returned to normal, although the blood pressure only partially recovered. This would correspond with the recovery of the heart as seen in electrocardiographic studies. The above changes are also in accord with the observation of Waddell and Cohen (23) who found evidence of decreased elasticity of the heart muscle when it was perfused with quinidine. The changes in the heart size noted above and indicated in figure 7 were produced by a two-fold mechanism, the peripheral action of quinidine producing a fall in blood pressure accounted for the contraction of the heart, and the direct action of the drug on the heart muscle in certain concentrations produced a dilatation. Both of these factors influenced the size of the heart simultaneously, but in an antagonistic manner, the resulting size was at any minute dependent on the relative degree to which one or the other was most effective.

The above experiments conciliate some of the conflicting views expressed by previous observers. There is positive evidence of heart muscle intoxication following quinidine. However, the respiratory paralysis cannot be explained by any other mechanism than a specific effect on the respirations and not as an indirect result of low blood pressure. Experience in the clinic with one patient who died following quinidine therapy and who showed respiratory embarrassment some hours before death makes it seem likely that means of resuscitation which proved so successful in these experiments might be applicable to human cases.

## CONCLUSIONS

1 As a result of experiments on cats the minimal lethal dose of quinidine bisulphate was found to be dependent upon the speed of administration Whereas 25 mgm per kilogram were usually fatal when given in a single dose on the other hand, when smaller doses were given at intervals of from six to twelve or twenty-four minutes, the total minimal lethal dose correspondingly increased to about 0.1 gram per kilogram

2 Immediately following injections of non-lethal doses of quinidine there was a striking fall in blood pressure This began a few seconds after the administration was started

3 The effect on the respiration was directly proportional to the size of the dose In giving a small dose the breathing was essentially unaffected, whereas after a moderate dose, there was usually a temporary, very brief cessation of the respirations With increasing sublethal doses there was a slowing of the rate and a decrease in the depth of the respirations With lethal doses the breathing gradually failed and finally ceased The heart always continued to beat after complete respiratory failure, for even as long as two minutes

4 The appearance of the animals under quinidine intoxication was not unlike that manifested by a fatal case in the clinic

5 It was found that when animals were given lethal doses and the respirations had ceased for a period of one to two minutes, they could be revived satisfactorily by artificial respiration combined with the use of caffeine sodium benzoate The caffeine alone was frequently sufficient, although artificial respiration alone was much more effective When the two procedures were combined recovery took place more rapidly

6 There were changes in the electrocardiograms of varying degree, depending on the size of the dose With small doses the changes were slight and transient With larger ones the curves indicated a grave intoxication of the heart muscle Fibrillation of the ventricle was very rarely observed

7 Roentgenograms taken at frequent intervals during the fall of pressure after an injection of a moderately large dose of quinidine showed at first a slight contraction of the heart followed by a dilata-

tion With small doses a contraction but no dilatation occurred There was a marked dilatation as a terminal event

8 It is suggested that the method of resuscitation employed in reviving the animals in these experiments may be applicable in the clinic in the treatment of quinidine intoxications

We wish to take this opportunity of expressing our indebtedness to Miss Bertha I Barker for her devoted assistance in these experiments

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most instances the recipients received 10 cc of blood each day except Sundays. The blood from the donors was obtained by heart puncture (10 cc from each animal) and except in a few instances to be mentioned later, the blood was kept from clotting by the addition of sodium citrate. Sufficient blood was obtained for all the transfusions of the day and mixed so that each recipient received pooled blood. Injections were made into the ear vein.

Before beginning transfusions the various bloods were tested for compatibility but in no case was incompatibility found. This is in accord with the results of Snyder (9). The hematological tests conducted were hemoglobin estimation (Haldane-Newcomer method), red and white blood cell count, reticulocyte count, and white cell differential count. The figures adopted as normal represent an average of three determinations obtained on different days before transfusions. After transfusion, the tests were repeated twice a week. The body weight was determined and serological tests of agglutination and complement fixation were made on the same days.

All the rabbits transfused developed a definite plethora, reaching a maximum in most cases in from 4 to 7 days. In two cases, however, the maximum was delayed until the second week. The degree of plethora varied in the different animals from 30 per cent to almost 100 per cent increase in the number of red cells and from 20 per cent to 50 per cent increase in hemoglobin. After the plethora had reached a maximum degree for the animal studied there occurred a gradual decrease in number of red blood cells and hemoglobin and this in spite of continued transfusions. In certain rabbits the number of red blood cells and percentage of hemoglobin decreased to about normal and remained normal, while in other rabbits the diminution continued until a true anemia developed. During the period of decrease in numbers of red blood cells and hemoglobin, the number of red blood cells usually showed a decrease before any decrease in hemoglobin became evident. Moreover, the curve representing the decrease in number of red blood cells usually took the form of a straight line, whereas the curve representing the decrease in hemoglobin was step-like. This is well shown in the curve representing these changes in rabbit 1 (fig. 1).

The reticulocyte count showed changes essentially like those first

demonstrated by Robertson. The number of reticulocytes varied inversely as the number of red cells. As the plethora increased, the reticulocytes decreased, in some instances vanishing entirely. With the appearance of blood destruction, the reticulocytes began to increase and in plethoric anemia the reticulosis was very marked. This phenomenon was very clearly demonstrated in rabbit 6 (fig 2)

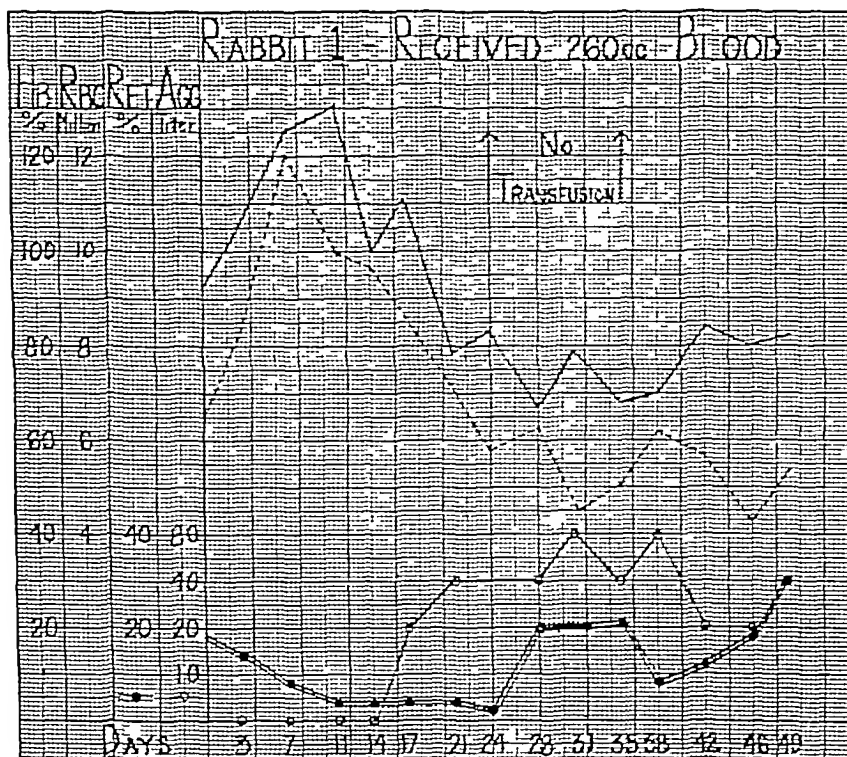


FIG 1

On the contrary, when no anemia developed, the reticulocytes showed merely the normal variations.

In only two instances did the white blood cells show an increase comparable to that of the red blood cells and hemoglobin. In the other rabbits, the number of leucocytes fluctuated irregularly above and below the normal.

During plethora the rabbits developed a definite lymphocytosis. With the anemia there was a loss in lymphocytes in favor of the neutrophils. Otherwise there was a good deal of fluctuation. Occasionally rarer forms of white cells were seen, but they were infrequent.

The body weight showed nothing abnormal. There was a good deal of increase and decrease, but these variations were not considered to

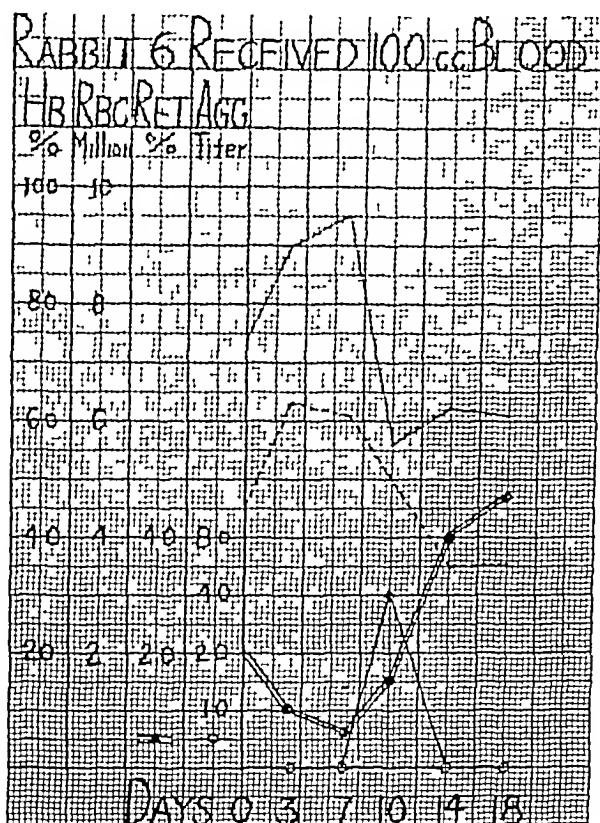


FIG 2

be of importance. Where the anemia was severe, as in rabbit 6, the body weight did show a definite gradual decline.

Certain of the rabbits failed to develop a very high degree of plethora and anemia and as it was thought that this might have been due to the transfusion of insufficient quantities of blood, one rabbit was transfused with 20 cc of blood each day, and a second rabbit with 30 cc of blood each day but the character of the curves representing the blood changes in both these animals do not differ from those rabbits

receiving 10 cc of blood per day. These two animals showed leucocyte increases commensurate to red blood cell increases.

As it was thought possible that the citrate present in the transfused blood might have an influence on the blood changes, we injected one rabbit daily with 10 cc of blood to which no citrate had been added. This rabbit, however, responded similarly to the other rabbits of this study.

Five of the transfused rabbits died and one was killed and these six rabbits were studied post-mortem.

The spleen usually showed enormous congestion. Microscopically hemosiderin was present in large amounts both intra and extracellularly. In some cases the congestion was sufficient to obscure the finer details of structure. The endothelial cells showed hyperplasia and the capillaries were loaded with cellular detritus and hemosiderin. An explanation for the lymphocytosis referred to was sought for in changes in the lymph follicles, but these appeared normal.

The bone marrow also showed marked accumulation of hemosiderin. A proliferation of blood cells had taken place and there was an extraordinary dilatation of the capillaries. In three instances an aplastic bone marrow was observed.

The liver showed a high degree of congestion and large quantities of hemosiderin, though neither was so marked as in the spleen and bone marrow. Each liver examined showed a varying degree of cloudy swelling and an intralobular infiltration of small round cells.

The lungs of two of the rabbits showed edema, congestion and partial atelectasis. The kidneys usually showed congestion and formation of casts. In the other organs no changes were observed, or only minor ones.

In an attempt to explain the mechanism of the blood destruction in the recipient rabbits, recourse was taken to a study of the development of iso-immune bodies active against rabbit red blood cells. Determinations were made for the presence of iso-agglutinins and iso-hemolysins in the blood of the recipient and later in extracts of various organs.

Agglutination tests were conducted using the recipient's sera against specimens of pooled washed red blood cells obtained from the various donors. Occasionally the sera of the recipients was tested against sus-



pensions of their own red blood cells and also against suspensions of each individual donor's cells. In making the suspensions in all cases the red blood cells were washed free of hemoglobin with saline and then suspended in saline in a 2 per cent concentration. One-half cubic-centimeter each of serum and cells were used. The tests were incubated for one hour at 37°C when tentative readings were made. Then they were allowed to stand in the ice chest overnight and a final reading was made on the following morning.

Hemolysin tests were made at the same time that agglutination tests were conducted. Undiluted recipient serum was tested against the same suspensions of red blood cells used for agglutination determinations. Guinea pig serum diluted 1:20 was used for complement after determining its hemolytic titer. Usually 1 cc of complement was used. Later, the recipient sera were used fresh and also inactivated, and unheated rabbit serum was substituted for the guinea pig complement. The tubes were incubated for one hour at 37°C and the readings were made both at the end of this period and on the following morning.

After the serum had been removed from the blood to be tested for the presence of agglutinins and hemolysins, the clot was washed in saline and then broken up in an amount of distilled water equal to that of the serum removed. This mixture was then centrifuged, the supernatant fluid rendered isotonic and used for agglutination and complement fixation determinations. The object of these tests was to determine whether antibodies might have been absorbed *in vivo* by the recipient's own red blood cells.

Agglutinins were demonstrated in the serum of only half of the recipient rabbits. In these instances, the highest titer was 1:80, and their presence was not constant. The appearance of agglutinins coincided with the beginning of blood destruction, but appeared to be in no way related to the degree of blood destruction. Rabbit 6, for example, though severely anemic, did not show as high an agglutinin titer as rabbit 2, in which animal no anemia developed (fig. 3). Similar results were obtained with the clot extracts.

The complement fixation tests were uniformly negative, regardless of the technique employed. This was true not only of the tests with serum, but also with the clot extracts.

Because of inability to demonstrate hemolysins *in vitro*, an effort was made to demonstrate their activity *in vivo*. For this purpose a rabbit was transfused in the usual manner until a plethoric anemia was induced. At this stage 10 cc of blood were withdrawn from this rabbit every other day and transfused into a second rabbit. It was impossible to continue these transfers for more than two weeks, but by that time it was obvious that blood from such a donor produced an

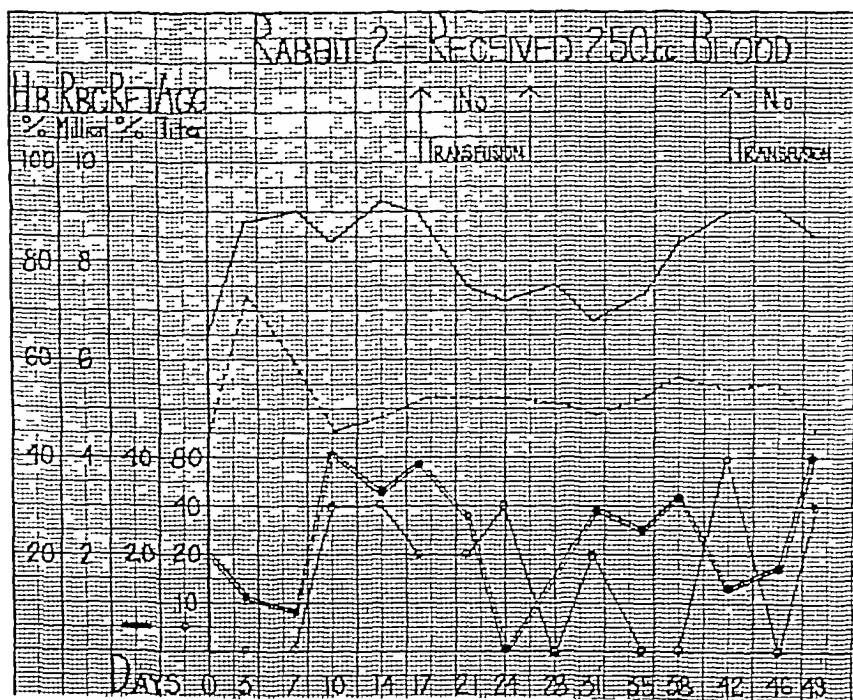


FIG 3

initial plethora in the recipient animal just as did blood from a normal donor (rabbit 8, fig 4)

The fixed tissues were then studied for their possible content in antibodies. Keyes (10) showed that in vertebrates red blood cell destruction is normally caused by fixed tissue phagocytes or hemophages. Later the same author (11) showed that a similar mechanism is active in the removal of foreign bodies. Cary (12) reported that

when foreign red blood cells are introduced into the blood stream, they are removed by hemophages. Motohoshi (13) also showed that rabbits remove foreign red blood cells by the hemophages of the spleen, liver and bone marrow, also that if colloidal silver is administered to animals the hemophages may destroy the animal's own erythrocytes

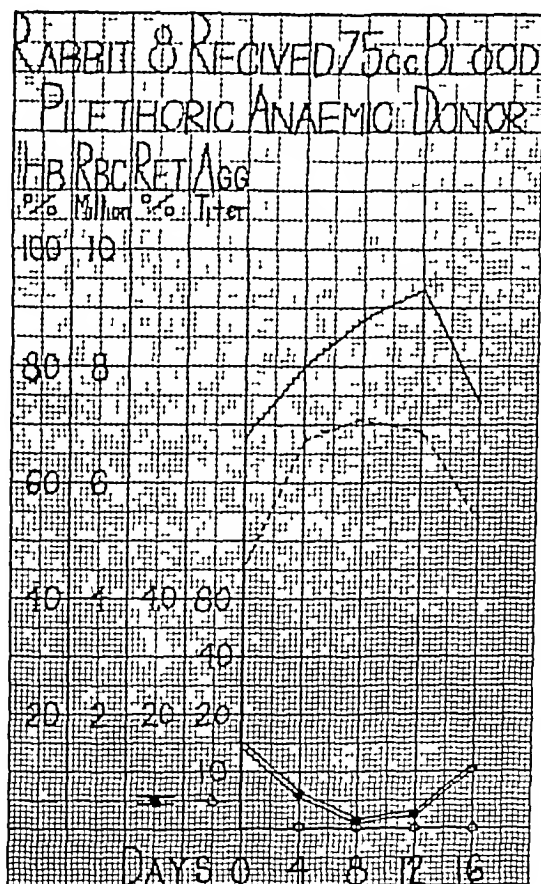


FIG 4

To determine the part played by fixed tissue immunity the following experiments were conducted. The liver, spleen, bone marrow and blood of transfused rabbits were ground individually in sterile mortars with the addition of small amounts of sterile sand and physiological saline solution. A portion of each of these mixtures was removed and employed for phagocytosis tests as will be described later. The grinding was continued and the material was further diluted about ten

times with saline. These emulsions were poured into sterile individual flasks and to each was added a few drops of chloroform and enough toluol to make a surface covering. The flasks were incubated for one week at 37°C and shaken several times a day. The extracts were then cleared by centrifuging and the supernatant fluid drawn off and tested for the presence of hemolysins and agglutinins. This method is essentially that of Cary's. In all cases the reactions were negative for agglutinins and hemolysins.

The portions first removed for phagocytosis tests were now mixed in capillary pipettes with equal quantities of suspensions of rabbit red blood cells (pooled blood). The pipettes were sealed and incubated at 37°C and samples were removed from the pipettes at the end of 15, 30 and 45 minutes, allowed to dry on slides and stained by Wright's stain. It was impossible to find any evidence of phagocytic or hemophagic activity in these preparations.

#### DISCUSSION

The chief problem in this investigation has been to explain if possible the mechanism active in the destruction of red blood cells following a plethora induced by repeated transfusions. The possible explanations considered have been (a) that the destruction is due to the development of iso-hemolysins, (b) that the blood destruction may result from increased activity of the hemophages or may be due to the appearance of an increased number of those cells.

As regards the second of these possibilities, no evidence was obtained to indicate that this mechanism is operative. A more thorough investigation was made of the first of these possibilities. It was impossible, however, to demonstrate any hemolytic activity of the serum of the transfused rabbits for mixed rabbit red blood corpuscles obtained from a group of rabbits. In view of certain observations of Ehrlich and Morgenroth and other observers, however, it seemed possible that while there might be found no hemolysins for the mixed cells of a number of rabbits, it might be possible to show that hemolysins active against the corpuscles of certain rabbits comprising a special group might be detected.

In 1900 Ehrlich and Morgenroth (14) reported that in goats following a single intraperitoneal injection of large amounts of goat blood

isohemolysins appeared but those were never active against the goats' own cells but were only active against the red blood cells of certain other goats. The authors concluded therefore that goats have potential hematological groupings which become real only under certain conditions of which their experiment supplied one, and these groupings therefore differ from those present in man where the groupings do not require any special stimulus in order to make them evident.

Hada and Rosenthal (15) in the case of chickens, Ottenberg, Kaliski and Friedman in dogs, Ottenberg and Thalheimer in cats with analogous methods were able to produce isohemolysins which, like the isohemolysins of Ehrlich and Morgenroth, were active against cells of certain individuals only. That a similar condition might be present in rabbits and that this might be related to the form of blood destruction being studied, the sera of the transfused rabbits were tested against suspensions of cells from a number of individual donors. Again, however, no hemolytic property of the sera could be detected.

In the sera of certain of the transfused animals moderate grades of agglutinating power for rabbit cells were found but cells from all donors were equally well agglutinated. In any case it is difficult to bring this property of agglutination into any causal relationship with the blood destruction.

Our experiments, therefore, do not supply any evidence suggesting a potential hematological grouping in rabbits and do not indicate that the appearance of iso-hemolysins in the blood of transfused rabbits is responsible for the blood destruction.

That antibodies might be formed by the fixed tissue cells and yet not be detected in the blood is still a possibility. Either all the antibodies might be fixed by the transfused corpuscles or the methods might not be delicate enough to detect the excess of antibodies present. If the former were the difficulty, it might be possible that antibodies could be detected if the tests were made only after a sufficient time were allowed to elapse following the last transfusion for all the foreign cells to be disposed of. This expedient yielded no different results, however. It is still possible that the rabbits' own red blood cells as well as those injected might bind the antibodies. To test this possibility an attempt was made to disrupt any possible union of antibody with the rabbit's own cells by extraction of the cells with water. This

procedure, however, also yielded only negative results. Finally, an attempt was made to detect whether antibodies were fixed to the tissue cells. With the methods employed no hemolytic antibodies were found.

#### SUMMARY AND CONCLUSIONS

1 Rabbits have been repeatedly transfused with rabbit blood. Plethora followed the transfusions and this in turn was followed by blood destruction.

2 The reticulocytes tended to decrease and in some cases to disappear with the plethora, but with beginning blood destruction, there was a rise in reticulocytes.

3 Leucocyte counts apparently bore no relation to the transfusions. The lymphocytes usually increased with plethora and decreased after a normal or anemic condition developed.

4 The striking feature of the pathology was the enormous deposition of hemosiderin in spleen, bone marrow, and liver. This was found both within phagocytes and extracellular.

5 Various hypotheses to explain the blood destruction following experimental plethora were tested by a number of methods. No evidence was obtained that the blood destruction is the result of the development of iso-hemolysins. No evidence was obtained to show that this result is due to greater activity of hemophages.

6 A number of facts concerning the blood changes occurring following transfusions have been obtained, but the explanation of post-plethoric anemia must await further work.

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# A STUDY OF THE EFFECTS OF PYLORIC OBSTRUCTION IN RABBITS

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## INTRODUCTION

In a recent paper Gamble and Ross (1) have demonstrated that, in the presence of pyloric obstruction in the dog, there occurs a loss in vomited gastric secretions of sodium as well as of chloride ion and water. They have shown the relationship of this loss of base to changes in the chemical structure of blood plasma and its significance from the point of view of the reparative action of injections of NaCl solution.

In this paper are presented measurements of the amounts of water, fixed base and chloride found in the stomachs of rabbits following experimental obstruction of the pylorus. These measurements were obtained with the purpose of determining quantitatively the extent of the loss into the stomach of each of these important components of the body fluids during the survival period following pyloric obstruction. Rabbits were used for the reason that in these animals the vomiting reflex is absent. Their stomachs will therefore conveniently collect for measurement substances leaving the body in the gastric secretions and contamination of this material by urine or feces is dependably avoided. It was found, however, that the collection period following obstruction could not be begun with an empty stomach. More than a week of fasting is required to completely empty a rabbit's stomach. The usual content is of a stiff texture and attempts to either wash it out or remove it surgically without considerably traumatizing the stomach were unsuccessful. The plan used in estimating



the loss into the stomach of water, fixed base, and chloride ion consisted in comparing measurements from control and operated animals. Fortunately the changes in the findings were of such magnitude as to render this method of approximate measurement satisfactory for the purposes of this study.

Besides these data demonstrating a rapid loss of blood plasma constituents, the results of a few analyses of muscle tissue and of skin are given which indicate a limited availability of material for correct repair of the plasma. The character of the actual resultant alteration of the acid-base composition of the blood plasma is illustrated by a few measurements of the concentrations of fixed base and of chloride and bicarbonate ions obtained from samples of blood serum.

ESTIMATIONS OF THE EXTENT OF LOSS OF WATER, FIXED BASE (B) AND  
CHLORIDE (Cl') INTO THE STOMACH FOLLOWING  
PYLORIC OBSTRUCTION

Measurements were obtained from the stomach contents of four controls (nos 1, 2, 3 and 4) and from four rabbits following obstruction of the pylorus (nos 5, 6, 7 and 8). The obstruction was produced by ligaturing the duodenum just below the pylorus with tape, the operation being carried out and the abdominal wound closed with aseptic precautions under ether anesthesia. In an additional experiment (rabbit 9) the obstruction was placed 40 cm below the pylorus, i.e., presumably at or somewhat beyond the lower end of the duodenum. The animals, except nos 8 and 9 which were found dead, were killed with ether, the operated ones as near the end of the survival period as could be judged. Two other rabbits (nos 10 and 11) were given intraperitoneal and subcutaneous injections of 0.9 per cent NaCl solution. Rabbit 10 received 250 cc intraperitoneally and 150 cc subcutaneously 24 hours and 29 hours respectively following operation. Rabbit 11 was given three intraperitoneal injections of 140 cc each,  $\frac{1}{2}$ , 18 and 25 hours after placing the obstruction. The former was killed with ether when obviously moribund and the latter while still alert and vigorous. The animals were placed in metabolism cages after operation. Food and water were withheld. The two that were given injections of NaCl solution voided small amounts of urine, the others were almost, or entirely, anuric. The rabbits did not

TABLE 1  
Measurements directly obtained from stomach contents and blood serum

Experiment	Animal number	Weight kilos	Survived operation hours	Stomach contents			Blood serum*		
				Solids grams	H <sub>2</sub> O cc	B cc 0.1 N	Cl <sup>-</sup> cc 0.1 N	B cc 0.1 N	HCO <sub>3</sub> <sup>-</sup> cc 0.1 N
Controls	1	2.1		21	89	68	151	169	20
	2	1.0		31	98	116	196	172	16
	3	2.3		20	67	64	100	[167]	22
	4	2.1		22	66	49	90	166	[22]
Average		2.8		24	80	74	131	169	20
Pylorus obstructed	5	3.0	30	17	313	376	162		10
	6	2.7	21	33	285	388	110	156	11
	7	3.8	36	21	320	313	510		
	8	2.0	27	13	225	267	358		
Average		2.9	29	21	283	341	113	156	10
Duodenal obstruction	9	2.7	32	35	315	613	399		
Pylorus of stricture, 100 cc NaCl solution given	10	2.8	18	28	722	928	1040		
	11	4.5	42	36	369	326	620	181	36

Additional data from stomach contents pH, Nos 5, 7, 8, and 10, 3.0, 3.7, 3.8 and 3.0 respectively  
 HPO<sub>4</sub> (as cc 0.1 M BEH<sub>3</sub>PO<sub>4</sub> following order in table), 8, 15, 14, 18, 16, 24, 21, 8, 36, 23 and 27

\* Values given are per 100 cc of serum. Those in brackets were obtained from other normal animals

vomit. It was planned to obtain a single blood sample from each of the animals but unfortunately, in the case of the operated animals, successful collection of a sufficient amount of blood for the three desired measurements near the end of the survival period was accomplished in only two instances.

The data obtained are given in table 1. It will be noted that the animals differed considerably in weight. The average weight of the

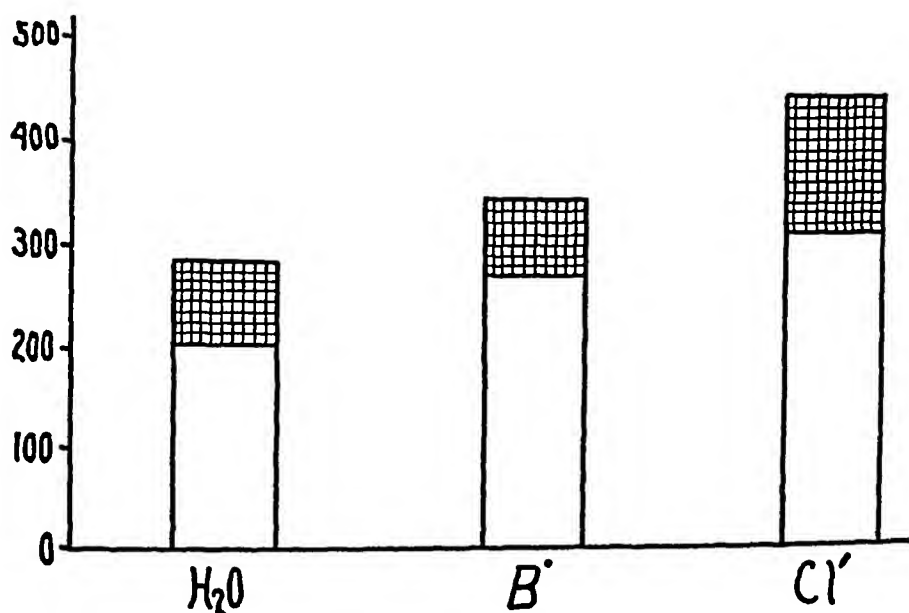


FIG 1 AVERAGES OF MEASUREMENTS OF H<sub>2</sub>O, B AND Cl' FROM STOMACH CONTENTS OF CONTROLS LAID OFF (CROSS-HATCHED PORTION OF THE DIAGRAMS) ON THOSE FROM RABBITS WITH OBSTRUCTION OF THE PYLORUS TO INDICATE EXTENT OF LOSSES INTO STOMACH FOLLOWING OBSTRUCTION

Measurements of water given in cubic centimeters and those of B and Cl' as cc of tenth normal solutions. Data from table 1

controls and of the four animals with obstruction placed at the pylorus was, however, almost the same. The averages of the measurements from these two groups demonstrate clearly that large amounts of H<sub>2</sub>O, B, and Cl' enter the stomach following pyloric obstruction. The extent of these losses is represented graphically by means of the diagrams in figure 1.

In table 2 are given the results of estimations of the extent of with-

drawal of H-O B Cl' from the body fluids following obstruction, in terms of the original total amounts of these substances in the blood plasma. These data except the values for plasma water, are derived from the averages of the measurements from the controls and from the four animals with obstruction at the pylorus, and from the single measurements from animal no. 9 in which the obstruction was placed at the lower end of the duodenum. The estimations of the initial quantity of water in the plasma were obtained by taking plasma volume as  $3.1 \times$  body weight, and water as  $0.92 \times$  plasma volume. The first of these factors was derived from Uthelm's (2) measurements of blood volume in rabbits by the dye method, and the second

TABLE 2

*Estimations of loss of water, fixed base, and chloride, following pyloric and duodenal obstruction in terms of original blood plasma content*

Experimental procedure	Pylorus obstructed	Duodenum obstructed
	cc	cc
Water lost	203	235
Initial plasma H-O	83	77
H-O lost — initial plasma H-O	2.4	3.1
	cc 0.1 N	cc 0.1 N
Fixed base lost	270	539
Initial plasma B	140	130
B lost — initial plasma B	1.9	4.1
Chloride lost	309	265
Initial plasma Cl'	85	79
Cl lost — initial plasma Cl	3.6	3.4

was directly determined in a plasma sample from one of the controls. The values for the total initial plasma content of fixed base and of chloride were computed from the estimated water volume and the average concentration of B and of Cl' found in the controls (see table 1). The extent of loss of each substance was estimated, as indicated in figure 1, by subtracting the average of the measurements from the controls from the average of the measurements obtained following obstruction. Obviously these data are roughly derived. They nevertheless serve to illustrate the quite startling rapidity of the losses of these three important body fluid constituents into the stomach by way of the blood plasma. As may be seen in the table, more than

twice the initial plasma content of water enters the stomach during the survival period following obstruction at the pylorus. Measurements of plasma volume in the presence of circumstances producing severe dehydration which have been reported (3) place the maximal reduction of volume at from 30 to 40 per cent. Depletion of plasma water to this extent would here represent only a fraction of the total loss of water.

Since a large reduction of ( $\text{Cl}'$ ) is seen in the plasma (table 1) it is not surprising that the loss of chloride ion into the stomach is found to be much larger than the loss of water, being 3.6 times the initial plasma content. As there is also a considerable reduction of ( $\text{B}$ ) in the plasma, a larger loss of fixed base than of water might be expected. The average estimated base loss into the stomach following pyloric obstruction is, however, less than the water loss. This discrepancy may very possibly be due to an additional loss of base secreted with relatively little water into the duodenum below the obstruction. The values for loss of water and of base obtained for animal no. 9 with obstruction at the lower end of the duodenum, excellently suit this surmise. It should be pointed out, however, that the above inferences based on concentrations observed in the plasma are not necessarily reliable since the chief source of these substances is not the blood plasma but the body fluids behind it.

As recorded in table 1, the survival period of these animals is short, being in average about 30 hours. Dogs live after this operation from 2 to 4 days. It has been thoroughly shown by Hartwell and Hoguet (4) and more recently by Haden and Orr (5) that dogs, after high intestinal obstruction, may be kept alive for a much longer period (2 to 3 weeks) if given repeated injections of physiological salt solution. The effect of administering  $\text{NaCl}$  solution to rabbits following pyloric obstruction was observed in animals nos. 10 and 11. In the case of no. 10 the first injection was not given until the morning following the day of operation. This animal became moribund at the end of 48 hours. Measurements obtained from the stomach contents and from a small amount of urine voided, given in table 3, indicate loss of all of the administered  $\text{H}_2\text{O}$ ,  $\text{B}$  and  $\text{Cl}'$ , with additional losses corresponding roughly to those found in the untreated animals. The degree of distension of the stomach in order to contain  $\frac{3}{4}$  of a

litre of fluid was extraordinary. The other animal (no 11) was given a first injection a short time after operation and two injections were given the next day. Whether it was because of the more suitable amounts and intervals of administration of salt solution can not be said, but this animal when sacrificed at the end of 42 hours was alert and vigorous. The measurements obtained (table 3) demonstrate a much less rapid dehydration than occurred in animal no 10 and a positive balance from the injected  $H_2O$ , B and  $Cl'$ . This, it may be incidentally noted, caused an actual increase of plasma (B) above its normal value which produced, in the presence of a usual

TABLE 3

*Estimations of balance for water, fixed base, and chloride, in animals given injections of NaCl solution following obstruction of the pylorus*

	Animal no 10			Animal no 11		
	H <sub>2</sub> O	B	Cl	H <sub>2</sub> O	B	Cl
	cc	cc 0.1 N	cc 0.1 N	cc	cc 0.1 N	cc 0.1 N
Found in stomach	722	828	1040	369	326	620
In stomach before obstruction*	80	74	134	98	116	196
Lost in stomach secretions	642	754	906	271	210	424
Lost in urine	45	70	5	70	110	9
Total lost	687	824	911	341	320	433
Given as NaCl solution	400	620	620	420	652	652
Balance	-287	-202	-291	+79	+332	+219

\* For this factor average of measurements from controls is used in the case of no 10. As no 11 was a very large animal, the data from no 2, the largest of the controls, was taken.

( $Cl'$ ), an increase of ( $HCO_3'$ ). These data suggest that, although the progress of dehydration is extremely rapid in rabbits following pyloric obstruction, frequent injections of appropriate amounts of salt solution would considerably prolong the survival period, at least until further distension of the stomach became impossible.

Haden and Orr (5, 6) have advanced the hypothesis that the benefit from injections of NaCl solutions following pyloric or upper intestinal obstruction is the result, not only of repair of dehydration, but also of a protective action exerted by chloride ion against a toxic substance assumed to be absorbed from the gastro-intestinal tract. They regard the chloride reduction in the plasma as only partially explained by

loss in gastric secretions since they find it in dogs in the presence of little or no vomiting and in rabbits which cannot vomit. They apparently derive the inference that the decrease in plasma ( $\text{Cl}'$ ) is in large part due to a withdrawal into the tissues and on this basis have devised the conception of a specific protective capacity of chloride ion which is supported by injections of  $\text{NaCl}$  solutions. The defect in the inference that, in the absence of vomiting, no appreciable loss of  $\text{Cl}'$  into the stomach can occur is apparent from the measurements

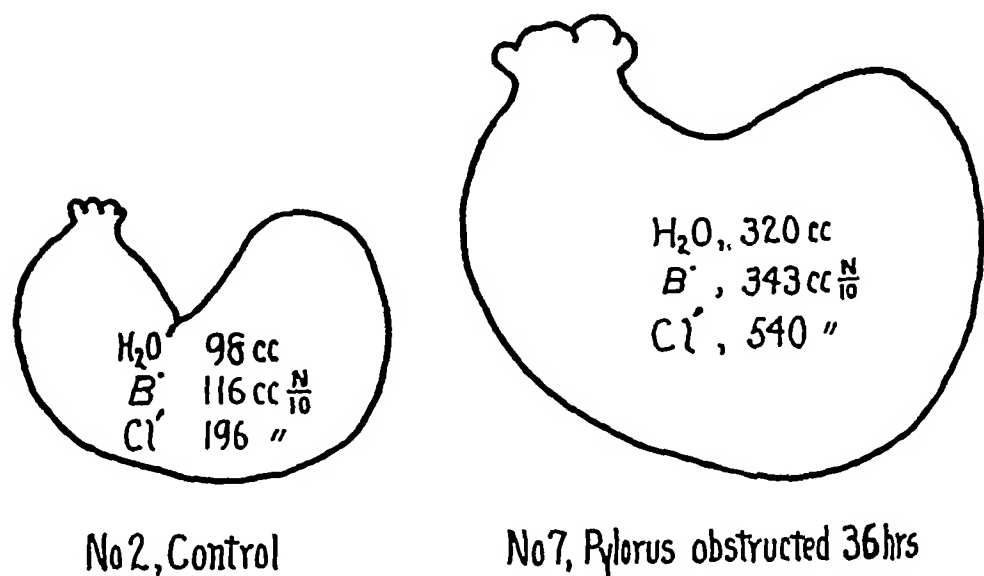


FIG 2 OUTLINE TRACINGS OF RABBITS' STOMACHS (ABOUT  $2\frac{1}{2}$  TIMES REDUCED) ILLUSTRATING GREAT DISTENSION PRODUCED BY RETAINED GASTRIC SECRETIONS FOLLOWING OBSTRUCTION AT THE PYLORUS

given above and is further illustrated by the diagrams in figure 2. It is clearly evident from the data in table 2 that the presence of any chloride at all in the plasma proves a rapid movement of  $\text{Cl}'$  in a direction quite the opposite of that surmised by these authors.

Obviously, however, the importance of the work of these investigators in thoroughly demonstrating the strikingly beneficial action of injections of  $\text{NaCl}$  solution in the presence of pyloric or upper intestinal obstruction is independent of conjecture offered in explanation of this effect.

## COMPOSITION OF THE FIXED BASE LOSS INTO THE STOMACH

The finding in this study which we believe is of chief interest is the large amount of fixed base entering the stomach. This loss, in proportion to the loss of  $\text{Cl}'$ , is larger than was observed by Gamble and Ross (1) in vomitus collected from a dog with pyloric obstruction. They found approximately  $\frac{1}{2}$  of the  $\text{Cl}'$  loss accompanied by Na. Using the estimations given in table 2, it appears that in rabbits the loss of B is more than 80 per cent of the equivalence of the  $\text{Cl}'$  loss.<sup>1</sup> We were at pains to demonstrate that, as might be expected, since the fixed base loss is derived from the blood plasma, it is composed almost entirely of Na. As may be seen in table 4, there was no

TABLE 4  
*Composition of total fixed base in gastric contents*

Experimental procedure	Animal number	Total base	Ca <sup>++</sup>	Mg <sup>++</sup>	K	Na <sup>++</sup>
		cc. 0.1 N	cc. 0.1 N	cc. 0.1 N	cc. 0.1 N	cc. 0.1 N
Control	1	68	3	4	20	41
	2	98	7	10	23	58
Pylorus obstruction	5	376	9	11	28	328
	6	388	10	7	17	354
Duodenal obstruction	9	613	10	11	100	492

\* Values for Na obtained by subtracting the sum of those for K, Ca and Mg from the total fixed base measurement.

appreciable increase in the amounts of Ca, Mg or K found in the stomach contents following pyloric obstruction. After duodenal obstruction a considerable increase of K was measured, but here also the chief loss is of Na. Obviously, owing to the unusual circumstances present, it is not permissible to infer from these data that Na is regularly a large factor in the construction and function of gastric secretions. That this may be the case is, however, suggested by the fact that Na was found to be the chief base in the stomach contents from the controls. Were the base here entirely food base, K should be present in largest amount.

<sup>1</sup> Besides  $\text{Cl}'$  a small additional inorganic acid factor in the stomach contents is indicated by the measurements of  $\text{HPO}_4''$  given at the foot of table 1.



MEASUREMENTS OF  $H_2O$ , B AND  $Cl'$  IN MUSCLE AND SKIN

It is evident from the data in table 2, that during the survival period following pyloric obstruction, plasma  $H_2O$ , B and  $Cl'$  are several times replaced. Maintenance of normal volume and composition will thus obviously demand a rapid movement of repair material from sources behind the plasma. It is desired here to compare briefly the probable extent of availability of these three chief components of the plasma. The loss of water as estimated in table 2, although more than twice the initial plasma volume, is in terms of the total water content of the body relatively small. Taking body water as  $0.70 \times$  body weight, the loss of water into the stomachs of the rabbits with obstruction at the pylorus may be estimated as 10 per cent of the initial body water. A certain additional loss must occur by way of the lungs and skin. On the other hand there is probably replacement of water to a considerable extent from oxidation of body fat, glycogen and protein. The conjecture is thus permissible that the extra-vascular water in the body can supply replacement of a relatively very large loss from the plasma without severely depleting the tissues. Obviously, however, the plasma cannot be reconstituted from water alone. Osmotic pressure factors will prevent a restoration of volume unless ionic content can also be rebuilt. Chloride ion and fixed base of the kind required in the plasma ( $Na$ ), are at hand to a very much less extent than water. Neither  $Na$  nor  $Cl'$ , the chief acid factor in plasma, are contained to appreciable extent in the largest compartment of extra-vascular water—the skeletal musculature. Presumably an important source from which these repair units may be derived is the skin where they are much more abundantly present, probably to a large extent in the interstitial fluid.

By way of partially illustrating the much smaller availability of  $Na$  and  $Cl'$  than of  $H_2O$ , the few measurements contained in table 5 are presented. These were obtained from samples of muscle and skin taken from one of the controls, no. 4, and from rabbit 8, 27 hours after ligation of the pylorus. The very slight extent to which muscle tissue contains the electrolytes required for plasma repair is shown by the measurements from the control animal. The determinations from the skin samples show much larger concentrations of  $Na$  and  $Cl'$  and

extensive contributions therefrom. It is of interest to note that base which will only slightly serve in reconstituting the plasma fixed bases is not appreciably withdrawn, as shown in the case of K and Mg, by an actual rise in the amounts found per 100 gram of skin accompanying the moderate loss of water. Haden and Orr (5) have shown some benefit from injection of hypertonic solutions of NaCl following pyloric obstruction. This finding they regard as demonstrating the specific protective action of chloride ion. It is however, consistent with the excess of availability of water over that of electrolytes here pointed out.

TABLE 5

*Data from analyses of muscle and skin from animals no 4 and no 8, demonstrating differences in extent of possible availability of plasma electrolytes from tissues*

Values per 100 grams	Muscle		Skin	
	Control	Pylorus obstruction	Control	Pylorus obstruction
Water, grams,	76.4	72.7	64.7	58.8
Cl, cc 0.1 \,	8.4	6.8	63.0	36.0
Total fixed base cc 0.1 \	121.0		119.0	103.0
Ca cc 0.1 \	2.4		6.5	6.3
Mg cc 0.1 \	12.3		6.4	7.2
K cc 0.1 \	103.0		34.4	36.6
Na * cc 0.1 \	3.3		71.3	52.9

\* Values for Na obtained by subtracting the sum of those for Ca, Mg, and K from the total fixed base measurement.

#### CHANGES IN PLASMA STRUCTURE FOLLOWING PYLORIC OBSTRUCTION

The few data in table 5 indicate that the losses from the blood plasma can produce but little alteration in muscle fluid composition. If it is the case that intra-cellular fluid elsewhere contains very little of the material required for plasma repair, cell composition in the vitally important tissues will be little affected by plasma depletions. It would thus appear that if these losses from the plasma lead to the death of the organism, this event must be referable chiefly to a failure of the function of the blood from disintegration of its chemical structure due to lack of replacement material rather than to coincident changes in the composition of the tissue cells. That the decline of

vital functions, in the presence of dehydration, may be attributed to impairment of circulation due to physical changes in the blood has been well argued by Marriott and his co-workers (7)

Regrettably, very few measurements of blood plasma values were obtained from the operated rabbits — These will, however, serve to

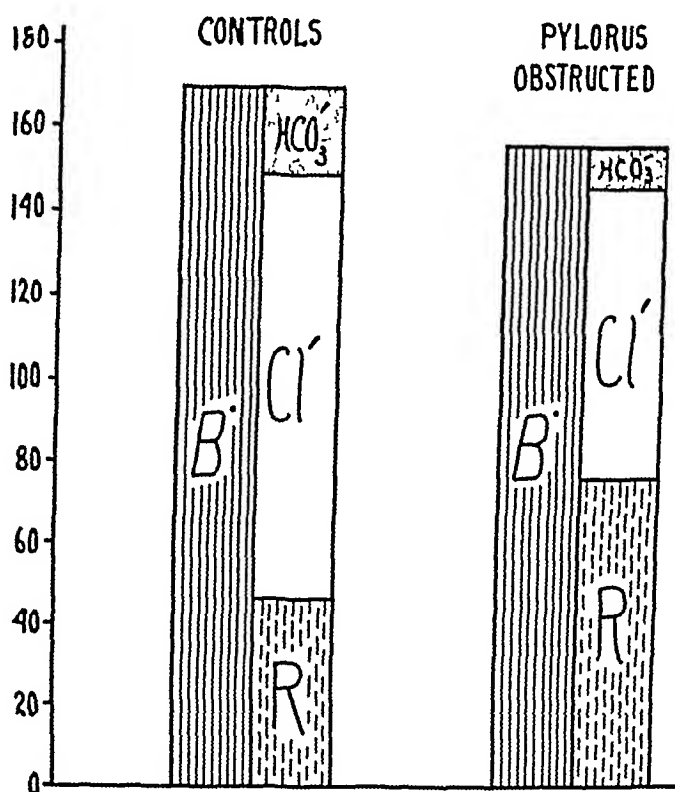


FIG 3 ILLUSTRATING CHANGES IN CHIEF FACTORS OF ACID-BASE STRUCTURE OF BLOOD PLASMA FOLLOWING OBSTRUCTION OF THE PYLORUS

Diagrams constructed from averages of measurements of  $B$ ,  $Cl'$ , and  $HCO_3'$  (as cubic centimeters 0.1 N per 100 cc of plasma) given in table 1.  $R$  represents the sum of the unmeasured acid factors, i.e.,  $HPO_4''$ ,  $SO_4''$ , organic acids, and protein

show the character of the changes in the ionic composition of the plasma which may result from the losses into the stomach. The averages of the measurements of ( $B$ ), ( $Cl'$ ) and ( $HCO_3'$ ) from the controls and of those from two of the animals after obstructing the pylorus, given in table 1, have been used in constructing the diagrams in figure 3. The measurements show, following obstruction, a reduction of all

three of the factors measured. The large decrease in  $(\text{HCO}_3')$  is perhaps surprising since in dogs and in man an alkalosis of some degree is nearly regularly found in the presence of obstruction at the pylorus. Gamble and Ross (1) have, however, pointed out that the increase of  $(\text{HCO}_3')$  which the reduction of  $(\text{Cl}')$  tends to produce is greatly limited by decrease of  $(\text{B})$  and by increase in some acid factor or factors as shown by increase of the sum of the individually unmeasured acid values designated as  $\text{R}$  in figure 3. The data in this paper show a much greater loss of  $\text{Na}$  in proportion to  $\text{Cl}'$  into the stomach than occurs in the dog, at least according to the measurements from the single experiment reported, and also an initially larger value for  $\text{R}$  in the plasma. As the diagrams indicate, the tendency of the loss of  $(\text{Cl}')$  to produce an increase of  $(\text{HCO}_3')$  was in these rabbits more than offset by reduction of  $(\text{B})$  and increase in  $\text{R}$ . As causes for this increase of  $\text{R}$  may be suggested an increased concentration of protein resulting from loss of plasma water and retention of other normal components of this fraction of total plasma acid due to the nearly anuric condition of these animals. It is not desired to suggest that rabbits following obstruction of the pylorus regularly die of an acidosis resulting from bicarbonate reduction although it is permissible to suppose that they occasionally may. The large reduction of bicarbonate in the plasma should, we believe, be regarded simply as one among many physical changes which together tend to impair the function of the blood.

#### SUMMARY

Following experimental obstruction of the pylorus in rabbits and during a short survival period, usually less than 30 hours, large amounts of water, fixed base, and chloride ion enter the stomach. These losses are, in extent, several times the initial total plasma content of  $\text{H}_2\text{O}$ ,  $\text{B}$ , and  $\text{Cl}'$ . Although rapid replacement occurs, correct plasma composition cannot be sustained. A much lower availability of the required electrolytes ( $\text{Na}$  and  $\text{Cl}'$ ) than of water, from elsewhere in the body fluids, is the limiting factor in plasma repair. The changes in the plasma which were observed in this study consist in a considerable reduction of  $(\text{B})$  and a larger recession of  $(\text{Cl}')$  which, however, is more than offset by increase of an undetermined acid

factor, or factors, with the result that plasma bicarbonate is greatly reduced. Plasma composition may be approximately repaired and the survival period considerably prolonged by repeated intraperitoneal (or subcutaneous) injections of NaCl solution. The losses from the plasma, however, continue and, in the rabbit, rapidly produce an enormous distention of the stomach.

The significance of a loss of body fluid is inadequately described by the term *dehydration*. Impairment of function following this event is referable not only to the loss of water, but also, and in larger degree, to the accompanying loss of particular electrolytes. An essential requirement in the treatment of dehydration is replacement of these ionic factors of body fluid structure.

The entrance into the stomach of a large amount of fixed base, more than three-fourths the equivalence of the chloride ion loss, constitutes a finding which is probably of important significance as regards the construction and function of gastric juice.

### *Methods used in obtaining measurements*

The gastric contents from the operated animals, which was largely fluid, was, after weighing, made up to a convenient volume in a stoppered graduated cylinder. As quickly as possible after thorough mixing an aliquot was taken and chloride determined in a sample from the supernatant portion. The remainder was dried on the steam bath, weighed, ground in a mortar, and a 2 gram portion nearly completely ashed by the Stolte method. The partially ashed material was extracted with four 10 cc portions of 0.5N HCl and, filtering through a small No. 40 Whatman paper, made up to a volume of 100 cc. Fixed base was determined in an aliquot from this extract. In the case of the controls the portion used for the chloride determination was measured by weighing, digested with about 10 times its weight of distilled water on the steam bath, and then made up to volume. The blood samples were obtained by cardiac puncture and the values given were determined in the serum. The muscle and skin samples were taken directly following death of the animals. Chloride was directly determined in 2 to 3 gram samples and fixed base in 15 to 20 gram samples ashed by the Stolte method and extracted with 0.5N HCl. The measurements of potassium, calcium, magnesium, and phosphorus given were also determined in the extracts of ashed material.

References to descriptions of chemical methods used are as follows: *Total Fixed Base*, Fiske (8), *Chloride*, Fiske (to be published), *Bicarbonate*, Van Slyke (9), *Potassium and Calcium*, Tisdall and Kramer (10), *Magnesium*, Briggs (11), *Phosphorus*, Briggs (12).

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# THE EFFECTS OF CHANGES IN HYDROGEN ION CONCENTRATION ON THE BLOOD FLOW OF MORPHINIZED DOGS

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## INTRODUCTION

The authors have recently studied the effects of partial respiratory obstruction on the respiration and circulation of the morphinized dog. In a previous communication (Blalock, Harrison and Wilson, 1925), it has been shown that when experimental respiratory obstruction is produced in dogs these animals develop a marked acidosis dependent on carbon dioxide retention and at the same time show an increase of from 40 to 200 per cent in the circulatory minute volume.

The object of the present study was to determine whether an acidosis independent of respiratory obstruction was accompanied by an increased blood flow, and, if this occurred, to observe the effect of the administration of alkali on the circulation in normal dogs and in dogs to which acid had been administered.

## METHOD

The technique, and the objections to the method used are discussed in detail in the former paper (Blalock, Harrison and Wilson, 1925). The procedure, in brief, was as follows:

Dogs were anesthetized with 0.06 gram of morphine, since it has been shown that this drug has relatively slight effect on the blood flow of normal dogs. The oxygen consumption was determined by a Benedict spirometer, with the graphic recording device. A specimen of blood was drawn from the right ventricle according to the procedure of Barcroft, Boycott, Dunn and Peters (1920). At the same time samples were taken from the carotid and femoral arteries.



One of the arterial samples was used for pH determination according to Hawkins' (1923) modification of Cullen's (1922) colorimetric method. In many of the experiments in which acid was injected slight laking of the blood occurred and accurate pH readings were difficult. Greater accuracy than  $\pm$  pH 0.03 is not assumed. However the changes found in H-ion concentration were much greater than the limits of error of the method.

The oxygen and carbon dioxide content of the arterial and venous blood were determined. The circulatory minute volume was calculated from the Fick formula

$$\frac{\text{cc O}_2 \text{ consumed per minute}}{\text{Amount O}_2 \text{ taken up in lungs by 1 cc of blood}} = \frac{\text{Number of cubic centimeters of blood flowing through lungs per minute}}{\text{cc of blood}}$$

Acid and alkali were injected into the femoral vein. In some experiments N/5 HCl was used, in others N/5, N/2.5 or N/1 lactic acid, N/1 and 2N Na<sub>2</sub>CO<sub>3</sub> were used. The amount of acid or alkali given was varied in different experiments, according to the size of the animal and the degree of change in pH desired. Fifty to 100 cc were injected in most instances. Some animals received acid alone, some alkali alone, while in a third group both acid and alkali were given. Five to ten minutes after the injection a second series of blood samples was taken. Forty-five to ninety minutes later a third series was withdrawn.

At the conclusion of the experiments the animals were autopsied, and the heart chamber examined. When a puncture in the intraventricular septum was found the experiment was discarded. In this way faulty results such as might have been obtained from mixing arterial and venous blood, were avoided.

## RESULTS

The animals were quiet and remained so throughout the experiments, with one exception (table 7). In some instances the respirations were very rapid whereas in others the breathing was normal or slowed. (It is well known that morphia produces an initial stimulation of the respiration of dogs.) The pulse was always slow at the beginning of the experiments. The animals were not cyanotic. The arterial and venous bloods were normally saturated with oxygen. The CO<sub>2</sub> contents were slightly elevated (42 to 54 volumes per cent) and the H-ion concentration slightly depressed (7.21 to 7.31). These are typical morphine effects. The control blood flows were between one



liter per minute, in the smaller dogs and two liters per minute in the larger animals

*The effects of acid* (Tables 1, 2, 3, 5, 6, and 7, figures 1 2, 3 and 4) As soon as the injection was made the respirations changed, becoming deeper in all instances and more rapid in some animals, slower in others (see fig 1) Thus hyperpnea rather than polypnea is characteristic of acidosis in the dog as well as in the human being The minute ventilation was increased in all experiments, the degree of increase varying from 30 to 200 per cent The effective ventilation was increased to a greater degree than the figures show because the deeper respirations after acid resulted in a smaller percentage of dead space exchange

A striking feature as one watched the dogs was the similarity in breathing to that seen in diabetic coma and uraemia These respiratory phenomena simulated closely those described in Walters' (1888) classical experiments

The effects on the pulse were variable, the rate being sometimes increased and in other instances diminished The oxygen consumption was not materially changed, though as a rule it was slightly increased One rather striking feature was the flushing of the gums and tongue This appeared within two or three minutes after the injection of acid and lasted twenty to thirty minutes This clinical evidence of vasodilatation is of considerable interest and is referred to later

The hydrogen ion concentration was uniformly increased, the degree of change varying greatly in different animals In general the increase was proportional to the amount and the rapidity of the injection of acid

The blood flow was increased in all instances, the degree of increase tending to parallel the degree of acidosis As this is the point which we wish particularly to emphasize the average figures for eight experiments in which acid was injected are shown

	pH	Blood flow per minute	Output per beat
		<i>liters</i>	<i>cc</i>
Before injecting acid	7.24	1.38	22.1
Five to ten minutes after the injection of acid	7.01	3.10	42.0
Forty-five to ninety minutes after the injection	7.17	2.01	30.2

## Showing the effects of the intravenous injection of acid

Experiment 12 weight 6.1 kgm	Pulse rate per minute	Respiration rate per minute	Oxygen consumption per minute	Arterial oxygen		Venous oxygen		Venous CO <sub>2</sub>		pH	Minute ventilation		Vol. time flow		Output per beat
				volumes per cent	cc	volumes per cent	volumes per cent	volumes per cent	volumes per cent		liters	liters	liters	liters	
Control period	54	23	61.7	21.1		18.1		18.5		7.20	2.2		0.94		17.9
Following injections of 60.0 cc N/5 HCl	50	32	79.1	21.1		19.1		31.2		7.00	1.1		3.11		13.0
Interval one half hour following injection	80	39	78.5	22.7		21.0		36.7		7.06	3.7		1.62		57.7

TABLE 2

## Showing the effects of the intravenous injection of acid

Experiment 11 weight 7.5 kgm	Pulse rate	Respiration rate	Oxygen consumption	Arterial oxygen		Venous oxygen		Venous CO <sub>2</sub>		pH	Minute ventilation		Vol. time flow		Output per beat
				volumes per cent	cc	volumes per cent	volumes per cent	volumes per cent	volumes per cent		liters	liters	liters	liters	
Control period	60	120	59.5	25.9		20.7		15.8		7.28	6.76		1.15		19.2
Following the injection of 50 cc N/5 HCl	60	101	62.6	21.5		21.5		36.7		7.11	7.51		2.09		31.9
Interval 30 minutes	18	60	65.1	25.7		21.2		41.3		7.16	6.80		1.15		30.2

TABLE 3

## Showing the effects of intravenous injection of acid

Experiment 10 weight 9.1 kgm	Pulse rate	Respiration rate	Oxygen consumption	Arterial oxygen		Venous oxygen		Venous CO <sub>2</sub>		pH	Minute ventilation		Vol. time flow		Output per beat
				volumes per cent	cc	volumes per cent	volumes per cent	volumes per cent	volumes per cent		liters	liters	liters	liters	
Control period	64	11	60.1	21.2		19.6		51.2		7.21	1.70		1.35		21.1
Following injection of 25 cc N/5 HCl	120	22	15.9	26.9		25.7		20.1		7.04	5.97		3.52		31.8

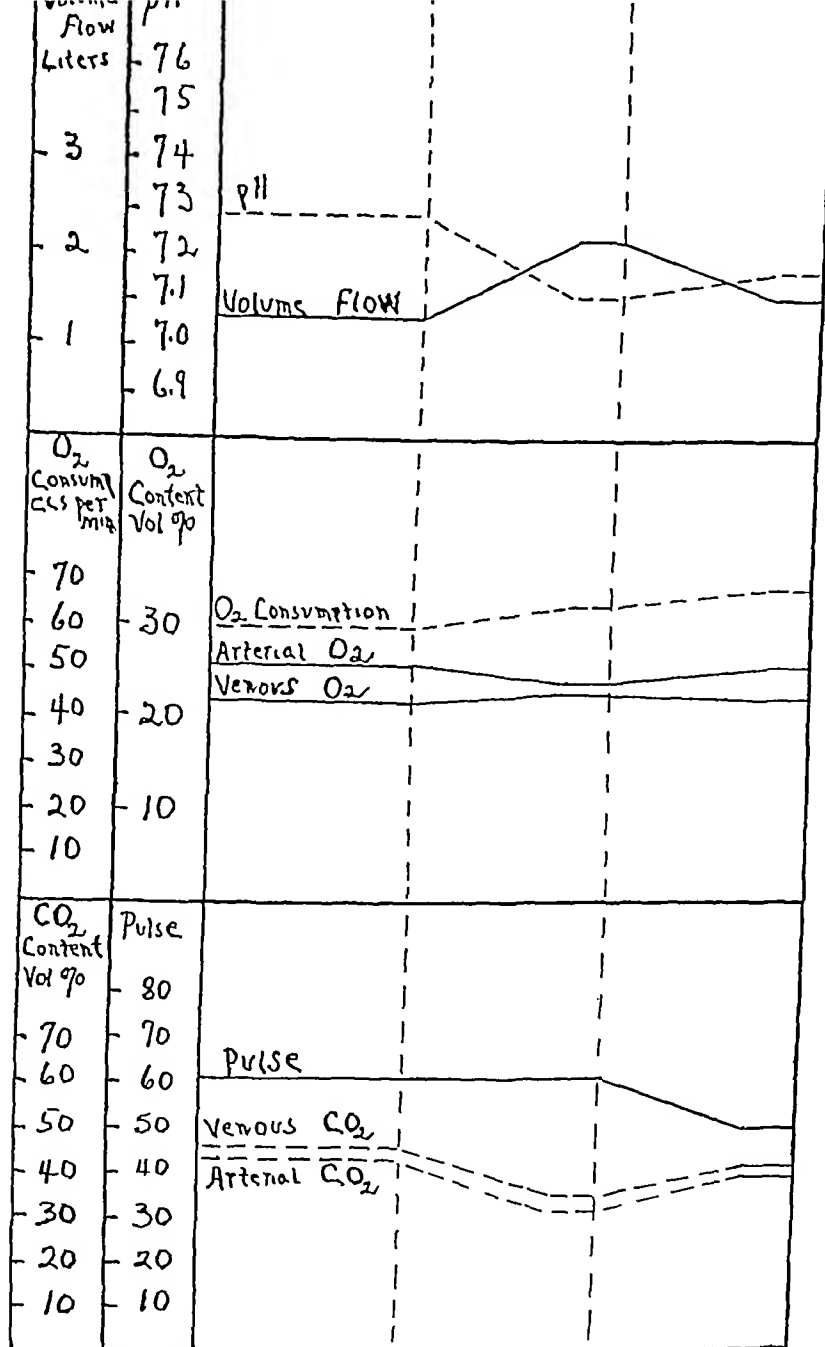


FIG 2 THE EFFECT OF ACID

This chart illustrates a typical experiment. Following the injection of acid the pH changed from 7.28 to 7.11 and the blood flow increased from 1.1 to 2.0 liters. The arterial and venous oxygen contents approached each other, the blood CO<sub>2</sub> content decreased. The pulse rate and oxygen consumption remained relatively constant. After 30 minutes the various functions approached normal.

In this and the following charts time relations are neglected. The various functions are drawn as if constant at the time of puncture. The drawings are purely schematic.

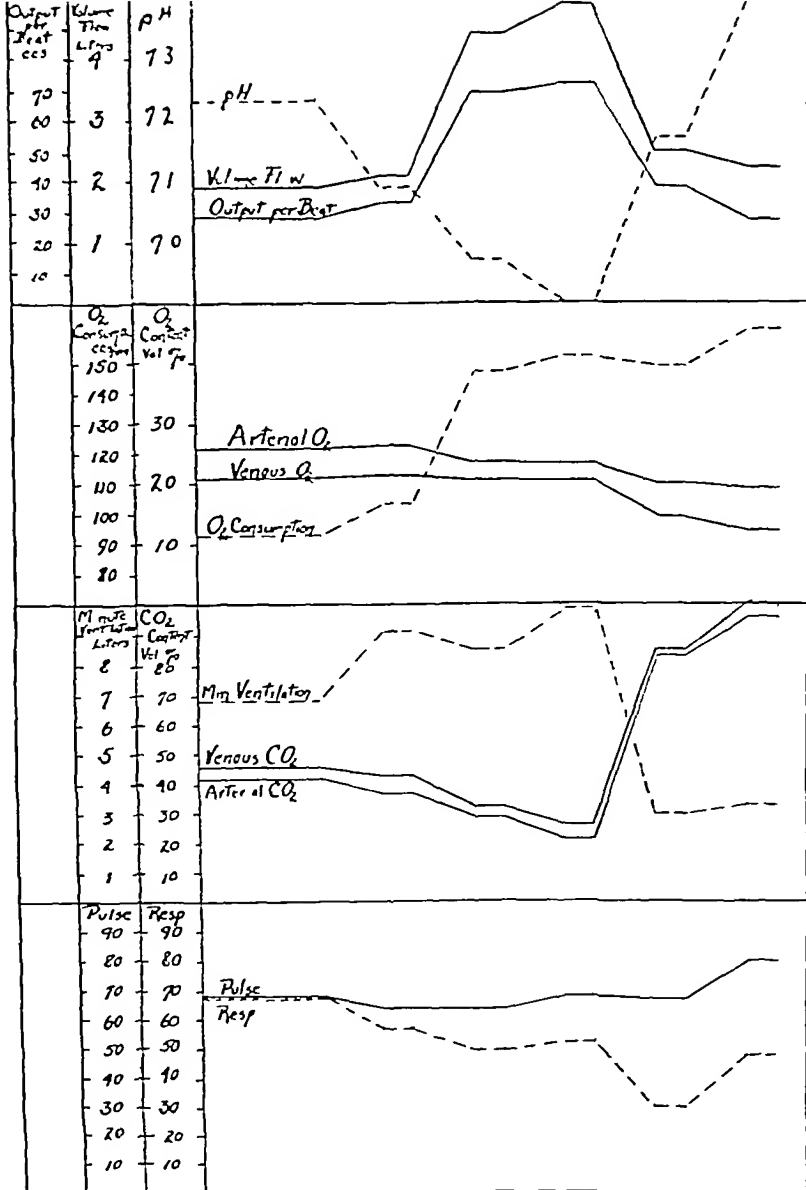


FIG 3 THE EFFECT OF REPEATED INJECTIONS OF ACID AND ALKALI

In this experiment the animal became restless and the oxygen consumption steadily increased. This probably explains the fact that even with alkalosis the blood flow was not less than in the control period. However the increasing blood flow with increasing H-ion concentration and decreasing blood flow with alkalosis were quite striking. The rise in minute ventilation and drop in blood CO<sub>2</sub> content with acid, and the reversed effects with alkali are noteworthy.

When acid was injected the blood flow was increased more than 100 per cent, and this increase was due to an increase in the output per beat rather than an augmentation of the pulse rate. After an interval of thirty to sixty minutes the acidosis diminished and the blood flow and output decreased.

The carbon dioxide content of the blood was, as would be expected, much decreased after the injection of acid, the figures varying between 10 and 30 volumes per cent. This fall was dependent on overventilation and was a compensatory mechanism similar to the changes in diabetic and renal acidosis.

*The effects of alkali* (Tables 4, 5, 6 and 7, figs 3, 4, 5 and 6) Almost immediately after the injection of 50 cc of N/1  $\text{Na}_2\text{CO}_3$  the respirations became very shallow and in many instances apnea occurred (fig 6). In only one animal was the apnea fatal. The animals did not become flushed but in several instances cyanosis was noted. The pulse rate usually increased by ten to thirty beats per minute. The oxygen consumption and arterial oxygen were not much affected, while the venous oxygen became distinctly lower. A very marked rise in both arterial and venous  $\text{CO}_2$  occurred. The H-ion concentration diminished, the blood flow and output per beat decreased. The average figures for four experiments were as follows.

	pH	Blood flow per minute	Output per beat
		<i>liters</i>	<i>cc</i>
Before injection	7.26	1.54	23.2
Five to ten minutes after the injection of sodium carbonate	7.60+	1.31	19.3
Forty-five to ninety minutes later	7.60+	1.19	14.5

It is to be noted that the H-ion concentration of the blood shows little tendency to return to normal. This is in contrast to the experiments with acid, and in which the pH values were approaching normal an hour after the injection. The difference is explained by the fact that the body can excrete acid by the lung as well as by the kidneys,

FIG 4 THE EFFECT OF ALKALI AND ACID

The usual effects of alkalosis, i.e., diminished volume flow, increased  $\text{CO}_2$  content are followed by changes in the reverse direction when acid is injected.





TABLE 4  
*Showing the effects of the intravenous injection of alkali*

Experiment 16 weight 7.5 kgm	Pulse rate	Respiration rate	Oxygen consumption	Arterial oxygen		Venous oxygen		Venous CO <sub>2</sub>		Arterial CO <sub>2</sub>	pH	Minute ventilation	Volume flow	Output per beat
				cc	volumes per cent	volumes per cent	volumes per cent	volumes per cent	volumes per cent					
Control period	72	88	67.6		18.5	14.2	37.3		36.4	7.31		8.19	1.57	21.8
Following the injection of 60 cc N/1 Na <sub>2</sub> CO <sub>3</sub>	84	42	72.5		17.8	13.7	67.9		61.8	7.60+		4.57	1.77	21.1
Interval 45 minutes	140	37	61.8		18.7	13.5	59.8		54.1	7.60+		5.37	1.19	8.5

TABLE 5  
*The effects of the intravenous injection of alkali following a previous injection of acid*

Experiment 14, weight 7.5 kgm.	Pulse rate	Respiration rate	Oxygen consumption	Arterial oxygen		Venous oxygen		Venous CO <sub>2</sub>		Arterial CO <sub>2</sub>	pH	Minute ventilation	Volume flow	Output per beat
				cc	volumes per cent	volumes per cent	volumes per cent	volumes per cent	volumes per cent					
Control period	60	120	59.5		25.9	20.7	45.8		42.7	7.28		6.76	1.15	19.2
Following the injection of 80 cc of N/5 HCl	60	104	62.6		24.5	21.5	36.7		37.0	7.01		7.81	2.09	34.9
Interval 30 minutes	48	60	65.1		25.7	21.2	41.3		38.3	7.16		6.8	1.45	30.2
Following injection of (1) 100 cc normal Na <sub>2</sub> CO <sub>3</sub> , (2) 60 cc N/1.5 Na <sub>2</sub> CO <sub>3</sub>	72	72	75.1		25.1	16.0	86.5		81.4	7.60+		3.53	0.83	11.5
Interval 25 minutes	60	40	68.6		26.0	17.9	76.9		70.9	7.58		3.72	0.85	14.1

TABLE 6

Showing the effects of the intravenous injection of acid folio line a previous injection of alkali

Experiment 17 weight 9.7 kgm	Pulse rate	Oxygen consumption per minute	Arterial oxygen	Venous oxygen	Arterial $\text{CO}_2$	pH	Vol ume flow	Output per beat
		cc	volumes per cent	volumes per cent	volumes per cent		liters	cc
Control period								
Following the injection of 50 cc N/1 $\text{Na}_2\text{CO}_3$	61	66.4	21.7	20.3	52.2	7.26	1.51	23.6
Interval one hour, injection of (1) 100 cc N/5 lactic acid, (2) 80 cc N/2.5 lactic acid	92	63	23.2	17.7	71.6	7.51	1.11	12.1
Interval one half hour, injection of (1) 100 cc N/2.5 lactic acid (2) 60 cc N/1 lactic acid	50	66.3	21.3	20.9	12.8	7.10	1.95	21.1
	80	71.7	23.7	20.2	17.1	6.90	2.11	26.7

TABLE 7

Showing the effects of repeated intravenous injections of acid and alkali

Experiment 18 weight 11.4 kgm	Pulse rate	Respiration rate	Oxygen consumption	Arterial oxygen	Venous oxygen	Arterial $\text{CO}_2$	pH	Minute ventilation	Vol ume flow	Output per beat
			cc	volumes per cent	volumes per cent	volumes per cent		liters	liters	cc
Control period										
Injection of 30 cc N/1 lactic acid	68	68	93.1	26.0	21.2	16.0	7.23	6.80	1.91	28.5
Interval 20 minutes, injection of 25 cc of N/1 lactic acid	64	57	101.2	26.5	21.7	13.3	7.09	7.12	2.17	33.9
Interval 20 minutes, injection of 30 cc of N lactic acid	64	50	137.3	23.8	20.7	32.1	6.97	8.50	1.13	69.2
Interval 35 minutes, injection of 50 cc of 2 N $\text{Na}_2\text{CO}_3$	67	52	112.0	23.3	20.1	26.1	6.90	9.85	1.90	72.1
Interval 20 minutes, injection of 35 cc of 2 N $\text{Na}_2\text{CO}_3$	80	30	135.9	20.0	11.6	85.3	7.17	3.00	2.57	12.8
		18	151.5	19.3	12.1	101.1	7.10	3	2.20	27.5

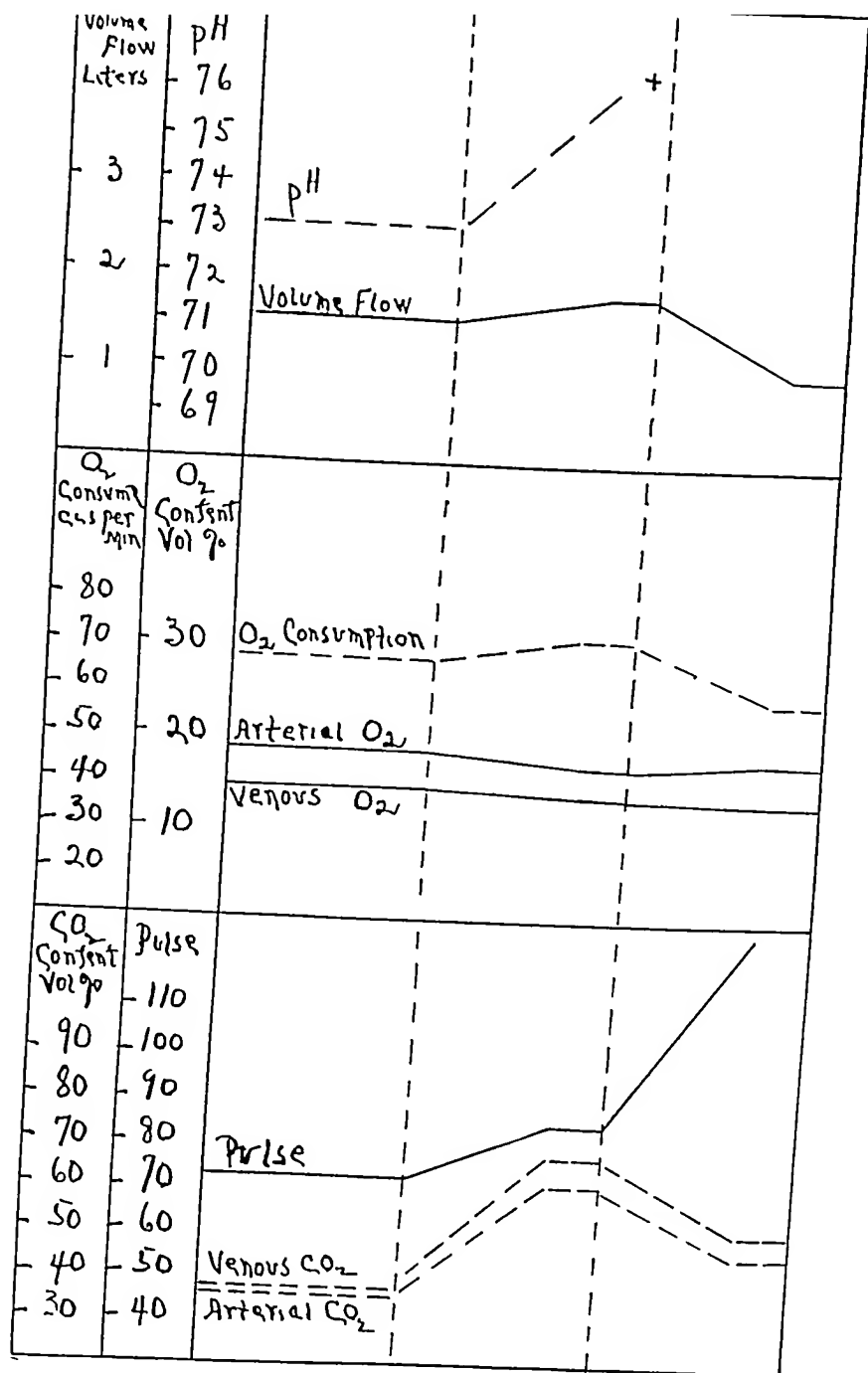


FIG 5 THE EFFECTS OF ALKALI

In this experiment alkali caused a slight initial rise in the blood flow, followed by a marked fall. The cause of the initial increase is not clear. The oxygen consumption was not changed. The pulse rate increased as the blood flow diminished. The usual elevation in CO<sub>2</sub> was found. A comparison of this drawing with figure 2 brings out the fact that the effects of alkali are more lasting than those of acid. This is discussed in the text.

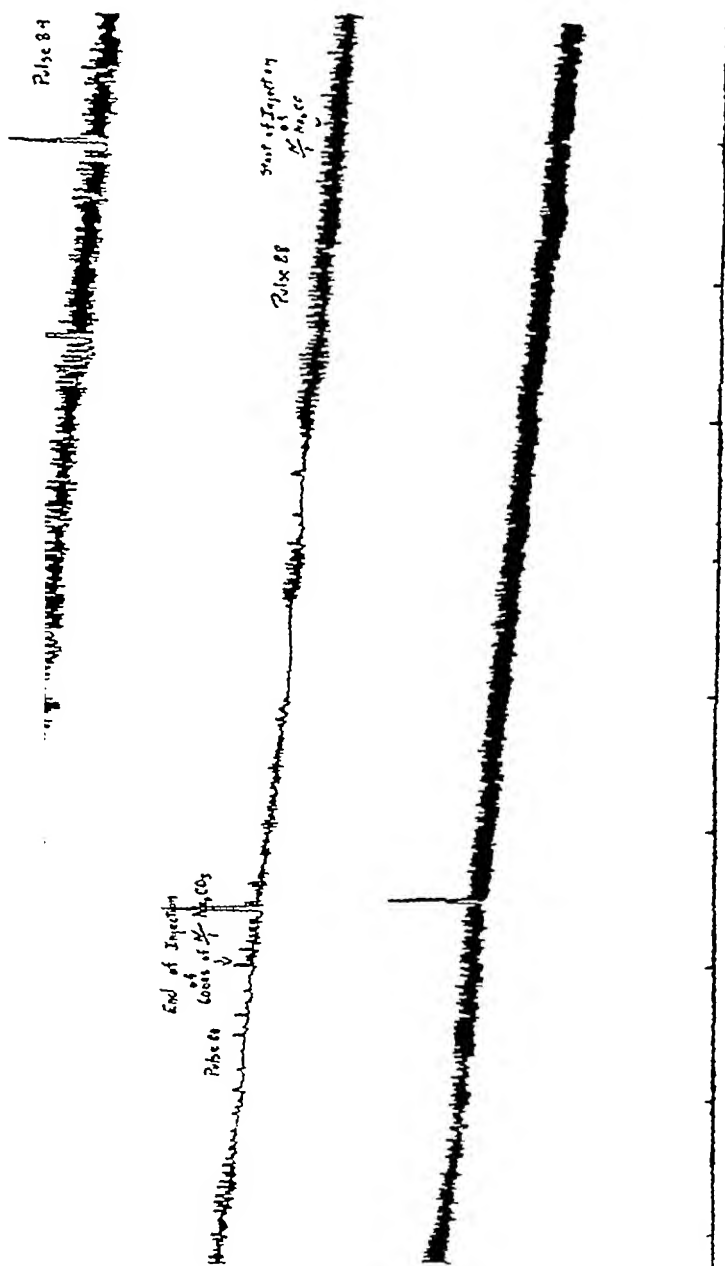


FIG 6 EFFECT OF ALKALI ON THE RESPIRATIONS

The curve passes from right to left and from below upwards. The decreased ventilation and short period of apnea after alkali are shown.

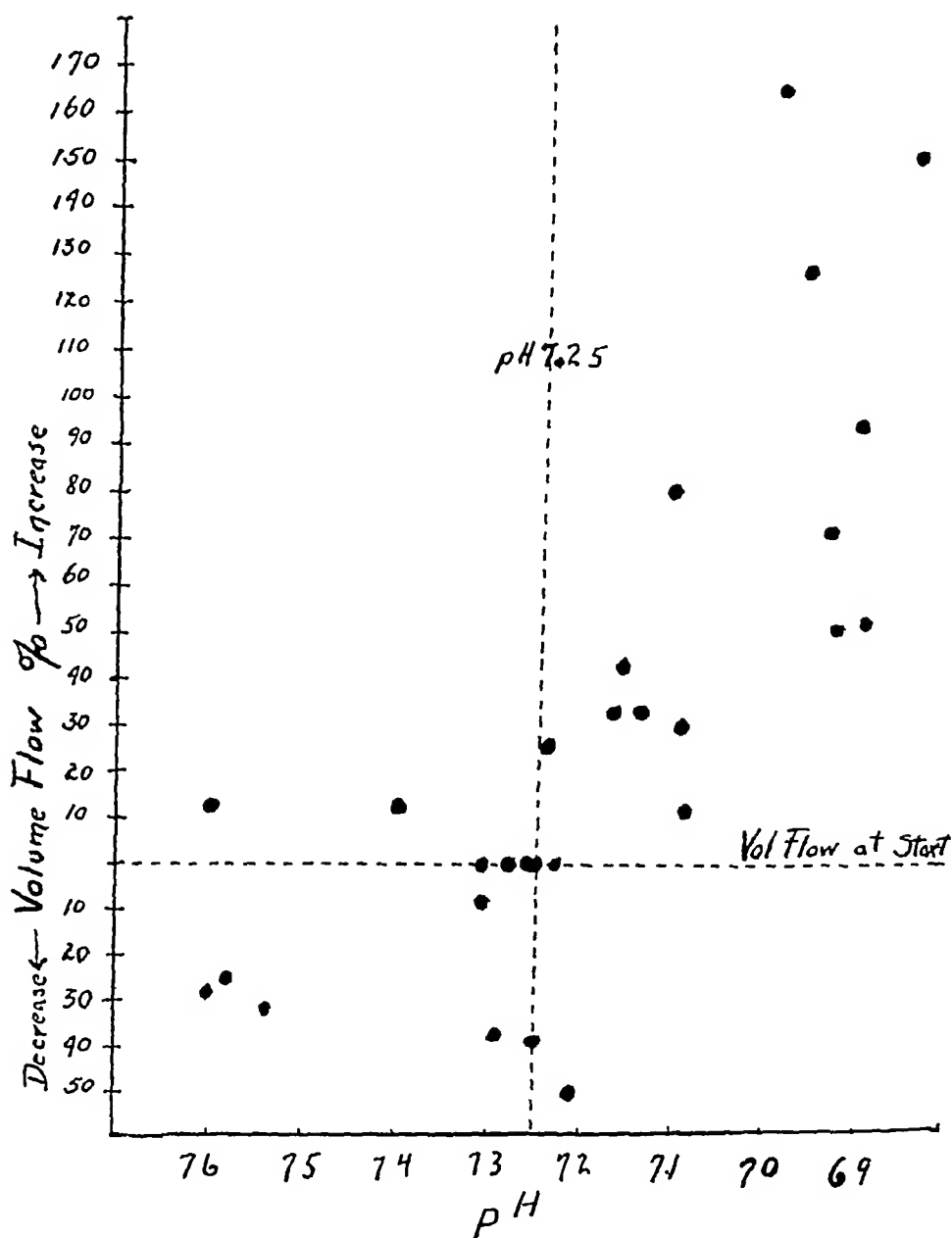


FIG 7 RELATIONSHIP OF CIRCULATORY MINUTE VOLUME TO HYDROGEN ION CONCENTRATION

Nearly all the dots to the left (alkaline side) of the normal pH line fall below the normal volume flow line, whereas the points on the right (acid) side are above the normal volume flow level

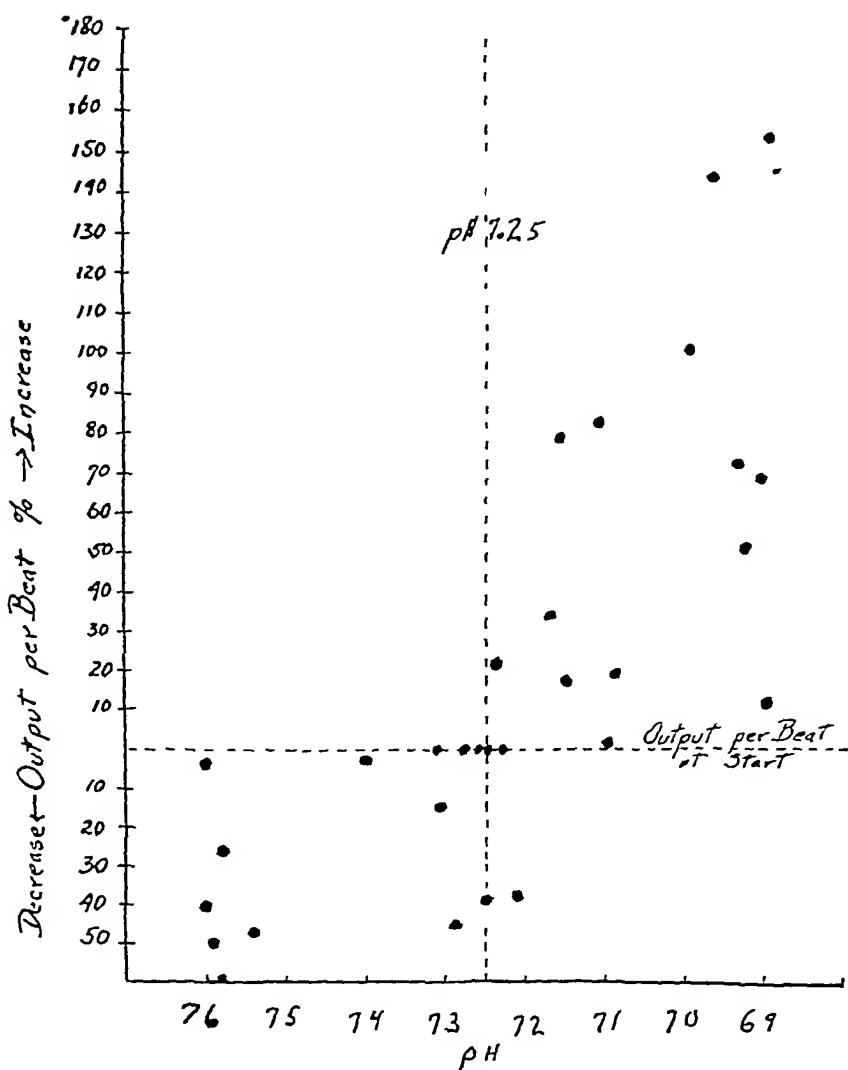


FIG 8 RELATIONSHIP OF OUTPUT PER BEAT TO HYDROGEN ION CONCENTRATION

The similarity to figure 5 brings out the point that the changes in volume flow resulting from changes in reaction, are due chiefly to variations in output per beat rather than in pulse rate

whereas excess alkali can only be excreted through the kidney. The blood flow became smaller and the output per beat diminished as the duration of the alkalosis increased. The more alkaline values for pH are denoted by 7.60+ because colorimetric readings with the Sorensen phenol red standards are unsatisfactory for dog's blood above this pH value.

In one experiment 40 cc N/10 sodium hydroxide was substituted for the usual sodium carbonate. The results were rather surprising. The breathing became at first slow and shallow but soon rapid and deep respirations were noted. At this point the pulse rate was 150. Blood samples were drawn and the animal was found to have an extreme acidosis (pH 6.90-), with a greatly diminished blood flow. A few minutes later death occurred—apparently from shock. This apparent paradox, the production of an acidosis by the injection of a strong alkali has been observed by others (McCallum, 1925) and can, we believe, be explained by the assumption that the caustic caused marked tissue destruction. The results of this atypical experiment are not included in the tables.

In figures 7 and 8, to which the reader's attention is especially directed, the results of our previous experiments on the acidosis produced by respiratory obstruction, are included as well as the changes in H-ion concentration found in the present series of experiments. In figure 7 the volume flow per minute is charted against the pH. It can be seen that acidosis, whether due to respiratory obstruction and hence associated with a high  $\text{CO}_2$  tension, or whether due to acid injections and thus associated with low  $\text{CO}_2$  tension, causes an increased blood flow (in the morphinized dog) whereas alkalosis is accompanied by a decreased flow. Figure 8 brings out the point that the changes in blood flow are due to variations in cardiac output and not to alterations in heart rate.

DISCUSSION<sup>1</sup>*1 The relationship of hydrogen ion concentration to blood flow*

Boothby (1915) first suggested that the H-ion concentration regulated blood flow. Douglas and Haldane (1922) have reached the same conclusion from the result of studies on man. Means (1924) in his recent monograph pointed out that this suggestion seems likely but, is, as yet, unproved. Henderson (1908, 1923) has repeatedly emphasized the importance of  $\text{CO}_2$  as a regulator of venous inflow and cardiac output.

In our experiments acidosis was associated with an increased circulatory minute volume, and alkalosis with a decreased blood flow (figs 7 and 8). It is of importance to know whether the change in volume flow is a result of the change in reaction or simply a concomitant phenomenon.

Part of the rise which occurred with acidosis might possibly be explained by the increased work of breathing. If the effect on volume flow were due entirely to the elevated metabolism of increased work a parallelism between blood flow and oxygen consumption should occur. No such parallelism was observed, for the oxygen consumption did not increase in as great a degree as did the blood flow. With alkalosis a conspicuous decrease in oxygen consumption occurred in only one instance whereas the blood flow was diminished in most instances. The exceptions to this were noted in the animals in which an elevated oxygen consumption occurred.

It might be said that the changes found, if not due to changes in work, were due to the mechanical effects of changes in the depth of respiratory movements with consequent variations in venous inflow.

<sup>1</sup> The literature on the subject of circulatory minute volume is extensive and is discussed in some detail in Henderson's (1923) recent review. Only such work as bears more or less directly on the relationship of H-ion concentration to blood flow will be noted here.

Most of the work previously done has been concerned with the effect of  $\text{CO}_2$  tension rather than the H-ion concentration, and has been done on animals anaesthetized with ether or urethane, both of which may very possibly modify the circulatory mechanism. Much of the work has been done on the isolated heart-lung preparation. Caution must be used in applying the result to the normal circulation.



This explanation does not hold in view of the work of Henderson (1908) who showed nearly 20 years ago that the mechanical factors are less important than the chemical factors. Furthermore, Marshall (1925) has found that the blood flow of the normal dog is no greater in summer than in winter, although the minute ventilations may be doubled because of panting.

Since therefore the changes in volume flow observed in these experiments cannot be explained on the basis of changes in metabolism or changes in the mechanism of respiration, we feel justified in stating that the variations observed in blood flow were due to the variations produced in H-ion concentration.

Eppinger (1924) has recently reported a great increase in circulatory minute volume during attacks of "cardiac asthma." Lewis and his associates (1913) observed acidosis during such attacks.

Patterson (1915), using the heart-lung preparation, found that high  $\text{CO}_2$  tension caused a diminished blood flow. The addition of adrenalin, however, to the perfusion fluid caused an increase. Adrenalin and  $\text{CO}_2$  caused a greater increase than did adrenalin alone. Cannon and Corrasco-Formiguera (1922) noted that the secretion of adrenalin was augmented by asphyxia. Thus one might expect an increased blood flow at a high  $\text{CO}_2$  tension.

Schneider and Truesdell (1922) found an increased flow through the hand when carbon dioxide in as high a concentration as 3 per cent was present in the inspired air. At higher concentrations the flow was diminished. Douglas and Haldane (1922) observed no increase in the circulatory minute volume when an excess of  $\text{CO}_2$  was breathed. They believed that no change in H-ion concentration was produced under the conditions of the experiments. Henderson and Harvey (1918) believe that a great increase in the circulation rate occurs when the  $\text{CO}_2$  tension of the blood becomes excessive.

Henderson (1923) emphasizes the importance of  $\text{CO}_2$  as a hormone regulating venous pressure, and hence the diastolic filling and circulatory minute volume. He points out that this effect of carbon dioxide may possibly be specific or may result from the increased H-ion concentration due to the  $\text{CO}_2$ . The latter hypothesis would seem to be the correct one in view of the above experiments. *Two different types of acidosis were produced, the one associated with a high  $\text{CO}_2$*

*tension of the blood, the other with a low CO<sub>2</sub> tension. In both conditions the volume flow was increased. Since the results were the same whether the acid was carbonic, hydrochloric or lactic, there is little doubt but that the increase in blood flow was due to the increased hydrogen ion concentration of the blood.*

Other observers (Henderson (1908), Douglas and Haldane (1922)), observed a decrease in blood flow with excessive artificial respiration, which as Grant and Goldman (1922) showed causes alkalosis. Under such conditions alkalosis is associated with a diminished CO<sub>2</sub> tension of the blood. In our experiments alkalosis was produced by the injection of alkali and was associated with a high CO<sub>2</sub> tension of the blood, but a decreased blood flow. Here again we are led to the conclusion that the changes in blood flow are determined by changes in the hydrogen ion concentration and not by variations in the carbon dioxide content of the blood.

It is of some interest to speculate briefly on the mechanism through which the pH influences the blood flow. The work of Fleisch (1921) who found that acid caused a peripheral dilatation and an increased capillary flow is of interest in this connection. Other factors remaining constant, capillary dilatation will, if not extreme, cause a more rapid return of blood to the veins, increased rate of venous flow and greater diastolic filling of the heart. It seems probable that this is the mechanism through which the H-ion concentration modifies the blood flow. Henderson (1923) expresses a similar idea when he discusses the importance of CO<sub>2</sub> in the regulation of venous pressure. Eppinger (1924) has evidence that the great increase in blood flow during attacks of "cardiac asthma" is due to diminished peripheral resistance. Harrison, Dock and Holman (1924) found an elevated blood flow in dogs with arteriovenous fistulae. This was probably dependent on diminished peripheral resistance.

## *2 The relationship of blood flow to output per beat*

One of the most striking results of the experiments was the finding of a 100 to 200 percentage increase in blood flow with no significant increase in pulse rate. This is contrary to Henderson's (1923) view, but is in accord with the finding of numerous other workers (Marshall (1925), Eppinger (1924), Zuntz and Hagemann (1898), Plesch (1909),

Krogh and Lindbard (1912) and others) In our experiments the pulse rate has usually paralleled the oxygen consumption more closely than the blood flow It is well known that the minute respiratory volume is controlled by two factors, the nervous factor and the chemical factor It is possible that the circulatory minute volume is similarly controlled

We have concluded from these experiments that the H-ion concentration constitutes the most important if not the sole regulatory mechanism of the circulation of the morphinized dog There is considerable evidence to indicate that this is also true in man However this evidence is by no means conclusive If sufficiently accurate methods are available this problem will be studied in the future At the present time no definite conclusions as to the circulation of man can be drawn from this work

#### SUMMARY AND CONCLUSIONS

The effects of acidosis and alkalosis in dogs have been studied with special reference to the blood flow Acidosis was produced (1) by obstruction to the respirations, (2) by the injection of hydrochloric and lactic acid Alkalosis was produced by the administration of sodium carbonate The changes in pulse rate, respiratory rate, minute ventilation, oxygen consumption, arterial oxygen, venous oxygen, arterial  $\text{CO}_2$ , venous  $\text{CO}_2$ , internal respiratory quotient, hydrogen ion concentration, circulatory minute volume and cardiac output per beat have been observed The following statements are believed to be true for the morphinized dog

- 1 An increase in the hydrogen ion concentration of the blood causes an increased blood flow

- 2 A decrease in hydrogen ion concentration causes a decreased blood flow

- 3 These effects occur regardless of the  $\text{CO}_2$  tension of the blood

- 4 Changes in the hydrogen ion concentration have no constant effect upon the oxygen consumption or the pulse rate

- 5 The cardiac output per beat is much affected by the hydrogen ion concentration, being greater than normal in acidosis and less than normal in alkalosis

6 The changes in respiratory rate are variable. When the respirations are unimpeded, intravenous injections of acid always cause increased respiratory depth and increased minute ventilation, and alkali usually causes diminished respiratory depth and diminished minute ventilation.

It is suggested that these results may be applicable to man.

It is a pleasure to acknowledge our indebtedness and express our appreciation to Dr E K Marshall, Jr, and Dr G A Harrop for encouragement and many helpful suggestions in regard to this work.

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PROCEEDINGS OF THE SEVENTEENTH ANNUAL MEETING  
OF THE AMERICAN SOCIETY FOR CLINICAL INVESTI-  
GATION HELD IN WASHINGTON, D C,  
MAY 4, 1925

*Cultivation of Vaccine Virus in Artificial Media* By ROBERT N NYE and (by invitation) FREDERICK PARKER, JR, Boston, Mass

Continuing the work of Parker it has been possible to cultivate vaccine virus in artificial media at 37.5°C for 130 days. The medium is essentially a living tissue culture, consisting of a small bit of normal rabbit testis to which is added infected testis (or plasma), the whole being held in place by one or two drops of diluted normal rabbit plasma. The cultures are made in small Petri dishes (5 cm diameter) and after coagulation of the plasma the dishes are inverted and sealed with sterile vaseline. Transplants are made every five or six days. Analysis of the air in the Petri dishes after five to six days incubation shows an oxygen content comparable to that of normal air. Intradermal titrations in rabbits show a marked increase in the actual amount of virus from generation to generation, thus eliminating the possibility of mere survival.

*Preliminary Observations on the Toxin of Streptococcus erysipelas* By H. L. AXOSS and (by invitation) KONRAD E. BIRKHAUG, Baltimore, Md

The sterile Berkefeld filtered urine of rabbits with experimental erysipelas and of patients early in the acute stage of erysipelas gives a reaction when injected into the skin of some normal rabbits and especially into rabbits having an active experimental erysipelas lesion. The skin-reacting substance is heat labile and neutralizable by anti-erysipelas serum and quickly disappears from the urine during recovery.

The toxin was produced by anaerobic growth of organisms in tryptic broth. One cubic centimeter killed rabbits weighing 1500 grams. Weaker toxic filtrates were concentrated by precipitation with  $(\text{NH}_4)_2\text{SO}_4$  at pH 4.6. The power of these streptococci to produce toxin does not parallel their hemolysin production. Anti-scarlatinal serum does not neutralize the toxin. Anti-erysipelas serum does not give Schultz-Charlton reaction in scarlet fever.

There is some evidence that erysipelas toxin is produced by a ferment elaborated by *Streptococcus erysipelas*.

Twenty rabbits received weekly injections of toxic filtrate either intravenously, subcutaneously, intramuscularly, or intracutaneously for four months. The serum of these rabbits acquired no antitoxic properties and the rabbits themselves were just as susceptible to intravenous and intracutaneous injection of toxic filtrate and of living erysipelas streptococci as normal rabbits. It appears that

the toxin in rabbits is not antitoxigenic and an analogy to the S factor of pneumococcus is suggested

*Observations on the Amount of Scarletinal Antitoxin Required to Cure Scarlet Fever*

By JAMES D. TRASK (by invitation) and FRANCIS G. BLAKE, New Haven, Conn

The goal to be attained in the treatment of scarlet fever with antitoxin is the neutralization of toxin and establishment of an excess of antitoxin in the blood of the patient. The amount of serum required will vary with the size of the patient, the severity of the disease, and the antitoxin content of the serum. To determine the amount of serum necessary the presence of toxin and antitoxin in the blood before and after intramuscular treatment has been determined in 42 cases of varying ages and severity, treated with 9 different lots of serum of varying antitoxin content.

The presence of toxin was determined by the capacity of the patient's serum to produce a local skin reaction in susceptible volunteers, the presence of antitoxin, by a positive blanching test. The antitoxin content of the serums was measured by determining the minimum amount of serum that would produce a positive blanching test.

The results show that a serum should contain at least 12,500 minimum blanching doses per cubic centimeter to be therapeutically efficient. This is equivalent to at least 10,000 skin test neutralizing doses per cc. or 100 units. Maximum doses are 3000 to 8000 units for children, 3000 to 12,000 for adults.

*Experimental Pneumonia in Mice Following Inhalation of Streptococcus hemolyticus and of Friedlander Bacillus*

By ERNEST G. STILLMAN and (by invitation) ARNOLD BRANCH, New York, N. Y.

The reaction of mice to inspired bacteria varies according to the kind of organism inspired.

Mortality and actual infection bear a direct relationship to the length of time the inspired bacteria persist in the lungs following inhalation. Hemolytic streptococci and B. Friedlander which have reached the lungs of normal mice following inhalation persist for several days. A fatal septicemia, with or without pulmonary localization, is frequent, and death may not occur for several days following exposure.

Pneumococci, on the other hand, rapidly disappear from the lungs of normal mice following inhalation and rarely cause a septicemia. In alcoholized mice, however, the pneumococci persist in the lungs for a longer period, a fatal septicemia is frequent, and pulmonary localization of the infection only occurs in partially immunized animals. Death occurs early, generally within 5 days.

*On Certain Pharmacodynamic Actions of Bacterial Poisons*

By KARL K. KOESSLER and (by invitation) JULIAN H. LEWIS, Chicago, Ill.

In the last ten years the senior author and his collaborators concentrated their attention on the chemical study of the poisons which are formed when micro-

organisms act upon proteins and amino acids. Quantitative chemical methods were devised which made it possible to separate these poisonous amines and to determine the amounts produced. It was shown that microorganisms which form histamine and tyramine are normal inhabitants of the human intestinal tract. Recent still unpublished investigations showed that this faculty of decarboxylation leading to amine production is not restricted to intestinal bacteria. The mixture of common pyogenic microorganisms contained in the bronchial expectoration, in tonsils and empyema fluids is able to form large quantities of these poisonous amines, which were pharmacologically characterized by their selective action on the smooth muscle fibre system. The question suggested itself naturally whether other poisons of similar constitution are not formed under our experimental condition?

This and other considerations made it desirable to study by delicate physiological methods bacterial culture filtrates and their various fractions obtained by chemical separation in the hope of elucidating, perhaps, the large problem of the factor of intoxication in bacterial diseases.

A method of determining bronchospasm in the guinea pig and a second method of demonstrating arterial constriction *in vitro* were devised. By means of these two methods the authors were able to show that the common microorganisms contained in the bronchial exudate, chiefly streptococci and pneumococci, produce chemically still unidentified poisons, which frequently have an intense constrictor action on the bronchioles and others which have definite vasoconstrictor action. The relationship of these experiments to human pathology (a) to bronchospastic forms of dyspnoea of non-allergic nature and (b) to the first stage of arterial hypertension, arterial spasm, is suggestive.

*Hay Fever. The Results of Treating the Same Patient in Four or More Successive Years.* By FRANCIS M. RACKEMAN, Boston, Mass.

A study of hay fever restricted to those patients with late hay fever due to ragweed, who were treated during several years, eliminates questions of pollen specificity and of widely different degrees of hypersensitiveness.

Skin tests done at the outset of each season show a remarkably constant degree of hypersensitiveness in 85 per cent of the 54 patients. The relation of the details of the treatment to the end result is best shown by curves which appear when the doses in each year are plotted against dates and amounts. The striking parallelism of these curves indicates again a degree of hypersensitiveness constant from year to year.

Similar courses of treatment lead to similar end results. Where however, the courses are different, it is possible to show that in some cases good results occur with a larger amount of treatment, while in other cases, excessive dosage, particularly if accompanied by large local or general reactions, leads to results less favorable than those in other years when the dosage was smaller.

This indicates that success depends upon a course of doses the amounts of which are optimal for the individual patient.



*Contribution to the Chemotherapy of Protozoal Infections* By GEORGE BAEHR, New York, N Y

Observation of cases of amebic colitis resistant to most drastic treatment with emetin hydrochloride and emetin bismuth iodide necessitated search for another agent which would not only destroy organisms within the intestinal lumen but would possess sufficient penetrating power to kill off amebae imbedded within the mucous membrane. The extreme sensitiveness of amebae to ultraviolet radiation suggested the utilization of secondary x-rays produced by Roentgen radiation of the abdomen after previous filling of the colon with fluorescent material.

An adequate agent seems to have been found in a combination of flumerin, a mercury fluorescein preparation invented by White as a spirochaetocide, and roentgen rays. Prompt and seemingly permanent disappearance of amebae was secured in chronic cases of years duration with rapid healing of ulcers and permanent disappearance of symptoms.

Intravital experiments in amebic colitis and in-vitro experiments with paramacia suggest that the generation of secondary x-rays within the intestine probably plays little if any role. The phenomenon appears analogous to that discovered over 25 years ago by v Tappeiner, who observed that the sensitiveness of protozoal organisms to fluorescent substances is enormously increased in the presence of sunlight. The possibility of a mercurial effect has not been excluded.

*Effects of Arsenic Injections on Endothelial Permeability* By WILLIAM F PETERSEN and (by invitation) T P HUGHES, CHICAGO, ILL

Therapeutic doses of arsenic (in normal dogs) produce a primary stimulation of endothelium. This is of short duration and seems largely confined to increased permeability for water. This is followed by a period of diminished permeability both for water and for larger protein aggregates (hemoglobin). We believe that this variation is partly responsible for the change of weight of patients under arsenic medication. With toxic doses the primary increase is not only for water but for colloids. Even in these cases there is a short period of recovery. Then follows a final period of increased permeability with other evidences of cellular injury (bile and erythrocytes in the lymph, as well as increase of sugar and phosphates). This final period seems to be irreversible and is the basis for the toxic manifestations of arsenic poisoning.

*Studies of the Capillaries and Thebesian Vessels of Human and Cat Hearts* By JOSEPH T WEARN, Boston, Mass

Intracardiac injections of India ink or dyes in living cats under various conditions have made possible the quantitation of the capillaries of the heart. A normally beating heart so injected may show an even distribution of 2000 to 3500 capillaries per square millimeter, and following the injection of histamine the number may be as high as 5700 per square millimeter.

*Cat heart after ligation*  
Capillaries per square millimeter

Left ventricle	Right ventricle	Pap. muscle	Sep. um	Auricle
5,728	5,616	4,700	5,614	4,800

These findings strongly suggest a reserve supply of capillaries in the heart. Perfusion of the coronary arteries was carried out and the quantity of outflow from all sources follows

Experiment	From Thebesian vessels of right auricle and ventricle	From left auricle and ventricle	Coronary sinus and veins	Leaks from cut surfaces
	“	“	“	“
1	250	235	50	80
2	142	100	37	47
3	235	175	20	120
4	200	183	8	70

In perfusing the coronary sinus and veins, all the perfusate escaped through the Thebesian vessels, while the perfusate through the Thebesian vessels escaped through the coronary sinus.

None of these procedures gave a good injection of the capillaries but injected larger vessels completely. Perfusion of the Thebesian vessels injected vessels similar to those injected when perfusing the coronary arteries.

All these findings hold true for a dead cat's heart and show the existence of a large direct connection between the coronary arteries and the chambers of the heart, in addition to the well known communication between the Thebesian vessels and the veins.

*The Effect of Caffem Sodio-Benzoate, Theobromin Sodio-Salicylate, Theophyllin and Euphyllin on the Coronary Flow and Cardiac Action of the Rabbit* By FREED M. SMITH and (by invitation) G. H. MILLER and V. C. GRABER, Iowa City, Ia.

Caffem sodio-benzoate, theobromin sodio-salicylate and theophyllin are generally believed to have a dilating action on the coronary vessels. More recently euphyllin has been said to augment the coronary flow to a far greater degree than either of the previously mentioned drugs. The investigation of the action of these drugs has resulted in varying results and in speculation on the part of the observer chiefly because of the methods employed and of the fact that the factors which influence the coronary circulation were not controlled.

The object of the present investigation was to determine the action of these drugs on the coronary vessels in concentrations that were estimated to approximate

that in man following the administration of a therapeutic dose and in experiments in which those factors influencing the coronary flow were as nearly as possible controlled. Furthermore, the effect of euphyllin, which has far greater action on the coronary flow than either caffeine sodium benzoate, theobromin sodium-salicylate or theophyllin was compared with that of nitroglycerin which is universally employed as a vasodilator.

*Experimental Coronary Embolism* By WALTER W. HAMBURGER and (by invitation) W. S. PRIEST, JR., and RALPH B. BETTMAN, Chicago, Ill.

The purpose of this investigation was to attempt to reproduce experimentally, chronic interstitial myocarditis in dogs by the injection of a suspension of lycopodium spores into the coronary circulation. This was carried out in a series of twelve dogs and the immediate and remote effects of this embolic obliteration of small arterioles and capillaries were studied electrocardiographically, at varying intervals, following the injection. After death gross and microscopic studies of the heart were made.

The results of this study may be stated briefly as follows. The pre-operative injection of morphin in dogs causes typical vagus stimulation effects. Ether narcosis in dogs is frequently followed by inversion of the T-wave and prolongation of the P-R interval. Relatively widespread and sudden obliteration of arterioles and capillaries from massive lycopodium spore injections results in immediate death of the animal, the electrocardiograph showing complete A-V dissociation, auricular flutter, or ventricular fibrillation. The injection of smaller amounts of lycopodium spores is compatible with complete recovery of the animal, the electrocardiograms in such dogs showing variously, inversion of initial ventricular complexes, right ventricular preponderance, nodal rhythm, ventricular extrasystoles, etc., with primary and secondary inversion of the T-wave. The apical areas of the hearts of these dogs show typical chronic interstitial myocarditis scarcely to be distinguished from that occurring in man as a result of atherosclerotic obliteration of the smaller coronary vessels.

*Experimental Heart Disease* By GEORGE R. HERRMANN, New Orleans, La.

The hearts of 200 normal dogs were divided by the author's midseptal line method and by Lewis' septum separation method. The averages for all the sectional and proportional heart weights for this control series were calculated. The most important ratios were as follows:

$$L/R^{(H)} 1.393, L/R^{(L)} 1.461, \frac{Ht}{Bd} - \frac{Wt}{Wt} 0.00798,$$

$$\frac{L}{Bd} \frac{V}{Wt} \frac{Wt}{Wt}^{(H)} 0.00369, \frac{L}{Bd} \frac{V}{Wt} \frac{Wt}{Wt}^{(L)} 0.00306$$

Experimental aortic insufficiency was produced in 75 of 150 operated dogs. Analyzed according to the time interval after operation (2 to 530 days) the maxi-

imum increases in the above ratios, respectively 47, 53, 47, 72, 75 per cent were found in the 70 to 110 day groups

Comparing the heart ratios found under various experimental conditions, it was evident that of the factors that determine the degree of hypertrophy, secondary infection and the relative youth of the dog are most important. Spartein and adrenalin, as well as thyroid feeding were not conspicuously effective. Pure traumatic lesions, especially in adults, produced very moderate changes. Evidences of heart failure were noted only in dogs with endocarditis. Spontaneous endocarditis developed in 6 pups under poor hygienic conditions, in 2 parturient bitches and apparently healed in 2 bitches who had had litters of puppies and 2 who had had no puppies and in 2 male dogs.

Electrocardiograms showed definite evidence of left preponderance in only one instance, that of the dog with the maximum cardiac hypertrophy obtained.

*Cinematographic Studies of Skin Capillaries in the Living Human Subject* By ALFRED E. COHN and (by invitation) J. HAMILTON CRAWFORD and H. ROSSENBERGER, New York, N. Y.

The method. The capillaries in the nail fold have been investigated, patients lie flat in bed with the arm stretched out horizontally at heart level. The apparatus consists of (1) lighting system with heat filter, (2) microscope, (3) stand for holding and adjusting finger, and (4) camera. The number of exposures used was ten per second. Room temperature varied from 19 to 22°C. The loops have been studied by projecting pictures, tracing their contour, and measuring the calibre at corresponding points (magnification 350).

Normal individuals, auricular fibrillation, mitral stenosis, chronic myocarditis and aortic incompetence were studied on consecutive days. Both limbs varied in calibre from moment to moment and from day to day in a similar manner. Changes were more marked and sudden variations occurred more frequently in cases of heart disease. Auricular fibrillation showed no dissimilarity from regular rhythm. These changes were uninfluenced by digitalization. They were unrelated to capillary contractility or individual heart beats. Some cases of aortic incompetence showed changes which suggested pulsation while others did not.

Blood flow varied in individual capillaries. In heart disease it was slower and more irregular. Digitalis improved it. Pulsation was sometimes seen in aortic incompetence. A film was demonstrated.

*Vasomotor Dilatation Following Sympathectomy* By LEONARD G. ROWNTREE and (by invitation) GEORGE E. BROWN, Rochester, Minn.

A group of cases in which periarterial sympathectomy and lumbar sympathectomy were performed were studied to determine the vasomotor and blood flow changes.

The first group included thrombo-angitis obliterans, Raynaud's disease, erythro-

melalgia and endarteritis obliterans in which the Leriche operation was performed. The second group included five cases in which the lumbar sympathectomy had been carried out for the relief of spastic paraplegia. The third group included one case of malignant hypertension in which lumbar sympathectomy was carried out with the hope of decreasing systemic arterial pressure and providing an area of diminished resistance which would dilate under strain and possibly protect the cerebral vessels.

The results of the Leriche operation were practically negligible with the exception of one case of Raynaud's disease in which demonstrable vasomotor dilatation was demonstrated by the hand calorimeter and skin temperature studies. Marked vasomotor dilatation takes place in the vessels of the legs following bilateral lumbar sympathectomy as shown by the calorimetric studies of the feet, skin temperature determinations and clinical evidence. There was also complete absence of sweating.

*The Effect of Various Factors on the Degree of Compensatory Hypertrophy of the Kidney after Unilateral Nephrectomy*<sup>1</sup> By LOIS LOCKARD MACKAY and EATON M. MACKAY (by invitation) and T. ADDIS, San Francisco, Cal.

One kidney was removed from albino rats of known age raised under constant environmental conditions. In an equal number of animals one kidney was exposed but not removed. Each day the body-weight and the consumption of a standard casein-starch-lard-vitamine diet adequate for growth and reproduction was measured. They were kept in a constant temperature room lighted through window glass. In measuring the effect of age 50 rats were used at each age period and in determining the effect of food changes 100 rats were used in each experiment. The standard or experimental diet was given for 14 days before the kidney was removed or exposed and for 40 days after. Then the animals were killed and the kidney weights obtained. Simple hypertrophy was measured by the percentage increase of the weight of both kidneys of the exposed group on the experimental diet over the weight of the kidneys in the exposed group on the standard diet. Compensatory hypertrophy was similarly measured by comparison of the percentage increase in the remaining kidney in the nephrectomised group over half the weight of both kidneys of the exposed group. Both forms of hypertrophy are expressed as per cent body weight.

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<sup>1</sup> This work was aided by a grant from the Ella Sachs Plotz Foundation.

Effect of	Simple hypertrophy	Compensatory hypertrophy
Age—females		
1 month		52 6
3 months		36 7
6 months		32 8
12 months		32 3
20 per cent urea diet (6 months—females)		
Control	0	33
Experimental	24	41
72 per cent protein diet (1 month—males)		
Control	0	37
Experimental	57	57
Acid diet—2 per cent $\text{CaCl}_2$ (1 month—females)		
Control	0	53
Experimental	15	53

*Blood Diazo Reaction in Cases with Impaired Renal Function* By G O BROWN and (by invitation) MARGARET RIGGS and O GARCIA, St Louis, Mo

Filtrates of blood plasma of nephritic cases after precipitation of the proteins with alcohol show a yellow color reaction when treated with Ehrlich's diazo reagent. The most marked reactions are seen in cases showing uremic symptoms.

The reaction is not given by uric acid, urea, creatine, creatinine, glucose, imidazole, phenyl alanine, guanine hydrochloride, guanidine hydrochloride, adenine hydrochloride, and adenine nucleotide. Negative results were also obtained with leucine, tyrosine, glycine, histidine, cystine, and the bile acids.

Sulfates, sulfites, thiosulfates, and thiocyanates do not give the reaction. Hydrogen and ammonium sulfide and organic substances containing an  $-\text{SH}$  group all give the reaction, e.g., ethyl mercaptan, thio-acetic acid, thiophenol, and cysteine. Carbon bisulfide, ethyl sulfide and ethyl disulfide, and diphenyl disulfide do not give the reaction.

Phenol, indole, and tryptophane also give the reaction.

While the identity of the substance responsible for the reaction in nephritic blood is not definitely established it would seem to be a type of substance whose retention has not yet been clearly shown.

*The Factors Causing Acidosis in Chronic Nephritis* By HAROLD A BULGER (by invitation) and JOHN P PETERS, New Haven, Conn

In a series of cases of chronic interstitial nephritis the inorganic constituents of the serum were determined in order to give some indication as to the cause of the acidosis. The total base was determined by Cullen and Robinson's adaptation of Fiske's urine method. Bicarbonate, chloride, phosphate and protein were also

melalgia and endarteritis obliterans in which the Leriche operation was performed. The second group included five cases in which the lumbar sympathectomy had been carried out for the relief of spastic paraplegia. The third group included one case of malignant hypertension in which lumbar sympathectomy was carried out with the hope of decreasing systemic arterial pressure and providing an area of diminished resistance which would dilate under strain and possibly protect the cerebral vessels.

The results of the Leriche operation were practically negligible with the exception of one case of Raynaud's disease in which demonstrable vasomotor dilatation was demonstrated by the hand calorimeter and skin temperature studies. Marked vasomotor dilatation takes place in the vessels of the legs following bilateral lumbar sympathectomy as shown by the calorimetric studies of the feet, skin temperature determinations and clinical evidence. There was also complete absence of sweating.

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<sup>1</sup> This work was aided by a grant from the Ella Sachs Plotz Foundation.

tory rate would increase the rate of elimination of carbon monoxide in asphyxiated animals. This was experimentally verified in asphyxiated dogs by keeping the pulmonary ventilation constant and treating with acids ( $\text{CO}_2$  and  $\text{HCl}$ ). The significance of this in therapy is discussed.

(2) The profound alteration of the normal oxygen dissociation curve affords an explanation of the anoxemia of carbon monoxide poisoning. For example, in a subject whose blood is 50 per cent saturated with  $\text{CO}$ , although the blood has a load of oxygen 2 to 3 times the normal physiologic requirements, its unloading tension is diminished to below 40 mm. At this tension the oxygen is relatively unavailable for tissue metabolism and the marked symptoms of carbon monoxide anoxemia follow.

*On Biliary System Function* By WILLIAM P. MURPHY (by invitation) and REGINALD FITZ, Boston, Mass.

This paper reports a comparison of the Rosenthal modification of the phenol-tetrachlorophthalein liver function test and the icterus index test in a series of fifty cases. The value of the icterus index as a practical and simple diagnostic test for disease of the biliary system is emphasized. A simple clinical method for determining the icterus index is described.

*The Significance of Bilirubinemia as shown by the Icterus Index* By ALICE R. BENNHEIM (by invitation) and NELLIS B. FOSTER, New York, N. Y.

A number of estimations in diabetes mellitus show the icterus index to be high in this disease. The high index is due to a hyperbilirubinemia. Carotinemia is ruled out, as determinations were made while the patients were on a three-day milk diet. (Carotin and xanthophyll disappear from the blood after a period of twelve hours.)

A number of sugar tolerance tests were made on normal individuals to see whether high blood sugar bore any relationship to the hyperbilirubinemia. The icterus index was seen to rise with the rise in blood sugar. The same test in diabetics showed a fall in icterus index with a rise in blood sugar.

The liver was stimulated by giving food, by arousing appetite, by liver function tests and by administering a choleagogue. Under these conditions a definite relationship was shown to exist between blood sugar and bilirubinemia. In diabetes a definite but different relation was disclosed.

The liver in diabetes is disturbed in its glycogen storing capacity. Glycogen is constantly being converted into sugar. Presumably this entails a hyperactivity on the part of the liver.

The hyperbilirubinemia in diabetes seems to be another manifestation of the hyperactivity of the liver cell in this disease.

*The Effects of Adrenalectomy and Adrenal Cortex Extracts in Muscular Fatigue*

By J. C. AUB, and (by invitation) WALTER BAUER, Boston, Mass.

The function of the adrenal cortex is unknown. There are two good ways of



determined by standard procedures to obtain some idea of the distribution of base among the various acids. If acidosis is defined in the terms of Van Slyke as a reduction of the bicarbonate content of the serum it appears that the acidosis of nephritis is caused by more than one factor. The molar increase of phosphate was insufficient to account for the total decrease of bicarbonate and chloride. A reduction of total base and increase of undetermined acids appeared to be the most significant factors, phosphate playing a somewhat less important role. Sulfate could hardly account for all the undetermined acid, presumably organic acids also played a part. The degree of acidosis appeared to be greatly influenced by variations of chloride. Frequently, especially with vomiting, chloride was quite low, thus allowing more base for combination with bicarbonate and resulting in only a slight reduction of  $\text{CO}_2$ . With high chloride, bicarbonate was sometimes extremely low.

*Acidosis Following the Feeding of 1-Hydroxystearic Ethyl Ester* By WALTER W PALMER and (by invitation) RANDOLPH WEST and ETHEL M BENEDICT, New York, N Y

This investigation was undertaken to attempt to determine whether or not an even carbon fatty acid with a negative group attached to the  $\alpha$ ,  $\gamma$ ,  $\epsilon$  or some corresponding carbon atom would yield acetone on oxidation in the body.

1-Hydroxystearic acid was prepared by the method of Saytzeff and esterified in the usual manner. The ester was then homogenized to a cream with skimmed milk. The acetyl value of the ester was 80-85. Three normal individuals were placed on a high fat diet and when excreting about 7 grams of acetone daily about half food fat was replaced by the synthetic product. The acetone excretion dropped to 3.5 grams daily, and stool fat analysis showed that the product fed was absorbed.

It is of interest to note that though the acetone falls, organic acid output remains almost unchanged, and it seems probable that the synthetic product is at least in part oxidized and not wholly stored by the body. In one experiment, not tabulated here, in which 1-oxyxystearic ethyl ester having an acetyl value of but 40 was fed there was no reduction in ketosis. It, therefore, appears that the non-hydroxylated fraction of the product fed is ketogenic.

*The Change of the Oxygen Dissociation Curve of Blood by Carbon Monoxide and Its Significance in Carbon Monoxide Poisoning* By WILLIAM C STADIE, Philadelphia, Pa, and (by invitation) KIRBY A MARTIN, New Haven, Conn

The effect of carbon monoxide upon the oxygen unloading function of blood is expressed in a mathematical equation theoretically deduced. Experiments *in vitro* on blood with varying oxygen and carbon monoxide tensions validate this equation.

By means of the equation the oxygen dissociation curve of blood, 50 per cent saturated with carbon monoxide, is plotted at a normal (7.4) and an acid (7.0) pH and contrasted with a normal curve.

(1) The curves predict that increase of acidity of the blood regardless of ventila-

normal individuals, even though the caloric intake, exercise, and mental state were inconstant. In the second group curves conforming to a single type occurred, blood and urine sugar being consistently elevated, but the urine showed much greater variations than the blood sugar. In the third group the change from the diabetic curves to normal curves as induced by Insulin was shown. In the fourth group a diagnostic relation between blood and urine curves was found in the mild and so-called renal diabetics. In the fifth group, which was studied on the supposition that recurrent furuncles might be associated with "peaks" of high blood sugar, usually missed when the specimen is taken with the patient fasting, the results were not conclusive.

The curves are of value as follows: (1) Aid in the prompt recognition of mild and so-called renal diabetics, under normal conditions of diet, as contrasted with the dextrose test meal. (2) Permit use of large doses of Insulin to obtain rapid desugarizing of patients, thus shortening hospital stay. (3) Furnish an accurate idea as to dosage, the optimum time of administration, and amount of Insulin necessary. (4) Permit the detection of cases of persistent hyperglycemia with negative urine.

*Frequently Repeated Blood Sugar Curves in Non Diabetic Individuals* By STANLEY COBB and (by invitation) WM. G. LENNOX, Boston, Mass.

From two to twelve blood sugar curves were done on each of 100 healthy or epileptic individuals. In a few of these individuals, the form of the successive curves varied markedly from time to time, but in most the form of successive curves was fairly uniform. In two thirds of the cases, repeated administration of glucose resulted in a progressive lowering of the sugar curve. On initial examination, about 30 per cent of the persons showed a "diabetic type" of curve. Of those who had one or more repetitions of the test, more than one half had normal curves on the second or third examination. If the administration of glucose was repeated after an hour or two, in case it was injected intravenously, the second curve was almost always slightly lower than the first. If the glucose was ingested, the second curves might be higher, lower or unchanged. On the basis of the 350 blood sugar curves made, we feel that a single observation of a high blood sugar curve, in itself, is without value. The observations suggest that in certain individuals the administration of glucose stimulates the glucose disposing mechanism of the body so that it handles subsequent doses of glucose more easily.

*The Action of Adrenalin Chloride on the Circulation in Man* By H. FIELD, Jr., (by invitation) and A. V. BOCK, Boston, Mass.

Despite observations in the literature that, under certain circumstances, at least, adrenalin causes vaso-dilator effects, the impression still prevails that it raises blood pressure by peripheral constriction. Our experiments on the rate of blood flow and other associated phenomena indicate that the main action of adrenalin is upon the output of the heart, which is greatly increased, and that no demonstrable peripheral constriction occurs.

investigating it after extirpation of the adrenal tissue (1) by finding an active cortical extract which will maintain life, or (2) analyzing physiological changes which occur. Experiments on the duration of life in cats have so far only increased our average from 32 hours in untreated animals to 55 hours in injected ones. Glucose improved marked adrenal insufficiency only temporarily.

Upon analyzing reactions of cats after adrenalectomy it seems obvious that they can make sudden movements with considerable force, but prolonged effort is impossible. We have therefore determined the amount of work individual muscles can do when left with an intact blood supply but pulling against a spring of known strength. About 17 hours after adrenalectomy such a preparation averages far less work than do normal controls. This offers a method for studying individual factors, also the effects of extracts upon this condition. Extracts which contain adrenalin increase contractions during the time of injection, but in preliminary experiments a cortical extract has given prolonged increased contractions for over twenty minutes. The correct active principle, however, should probably do this for several hours.

We are continuing these experiments.

*Studies Bearing Upon the Composition of Rachitic Bone* By W. McK. MARRIOTT, B. KRAMER and J. HOWLAND, Baltimore, Md.

From the analyses made upon the bones of children and of rats, it may be said that the calcium phosphate compound in all bones, rachitic and non-rachitic, is tricalcium phosphate. It is possible to show that the proportion of calcium phosphate to calcium carbonate is greater in the bones of the normal than in the rachitic rat. Analyses of human bones show that this is also true of children.

Precipitates made from solutions comparable in their inorganic composition to human serum show that these precipitates contain essentially the same calcium phosphate compound as bone and that the ratio of calcium phosphate to calcium carbonate, other conditions being the same, varies directly with the inorganic phosphorus content of the solution just as do the bones of rachitic and normal animals. This must not be taken to indicate a belief that calcification is a simple precipitation. There are other factors that must be taken into account.

*Observations on the So-called Occult Urine Sugar with Particular Reference to the Blood Sugar Level* By L. W. GORHAM, THOMAS ORDWAY and (by invitation) FRANK T. HUESTED, ALBANY, N. Y.

Specimens of blood were examined every two hours by the micro method of Randles and Grigg, and two hourly specimens of urine were examined for their sugar content by the Benedict-Folin-Hawk Method.

Curves were charted in series of cases represented in the following groups: (1) normal individuals, (2) Untreated diabetics, (3) diabetics under insulin, (4) so-called renal diabetics, (5) staphylococcus skin infections.

In the first group characteristic normal curves were determined on varying diets. Average normal curves were made which showed only slight variations in different

normal individuals, even though the caloric intake, exercise, and mental state were inconstant. In the second group curves conforming to a single type occurred, blood and urine sugar being consistently elevated, but the urine showed much greater variations than the blood sugar. In the third group the change from the diabetic curves to normal curves as induced by Insulin was shown. In the fourth group a diagnostic relation between blood and urine curves was found in the mild and so-called renal diabetics. In the fifth group, which was studied on the supposition that recurrent furuncles might be associated with "peaks" of high blood sugar, usually missed when the specimen is taken with the patient fasting, the results were not conclusive.

The curves are of value as follows: (1) Aid in the prompt recognition of mild and so-called renal diabetics, under normal conditions of diet, as contrasted with the dextrose test meal. (2) Permit use of large doses of Insulin to obtain rapid desugarizing of patients, thus shortening hospital stay. (3) Furnish an accurate idea as to dosage, the optimum time of administration, and amount of Insulin necessary. (4) Permit the detection of cases of persistent hyperglycemia with negative urine.

*Frequently Repeated Blood Sugar Curves in Non Diabetic Individuals* By STANLEY COBB and (by invitation) WM. G. LENOX, Boston, Mass.

From two to twelve blood sugar curves were done on each of 100 healthy or epileptic individuals. In a few of these individuals, the form of the successive curves varied markedly from time to time, but in most the form of successive curves was fairly uniform. In two thirds of the cases, repeated administration of glucose resulted in a progressive lowering of the sugar curve. On initial examination, about 30 per cent of the persons showed a "diabetic type" of curve. Of those who had one or more repetitions of the test, more than one half had normal curves on the second or third examination. If the administration of glucose was repeated after an hour or two, in case it was injected intravenously, the second curve was almost always slightly lower than the first. If the glucose was ingested, the second curves might be higher, lower or unchanged. On the basis of the 350 blood sugar curves made, we feel that a single observation of a high blood sugar curve, in itself, is without value. The observations suggest that in certain individuals the administration of glucose stimulates the glucose disposing mechanism of the body so that it handles subsequent doses of glucose more easily.

*The Action of Adrenalin Chloride on the Circulation in Man* By H. FIELD, Jr., (by invitation) and A. V. BOCK, Boston, Mass.

Despite observations in the literature that, under certain circumstances, at least, adrenalin causes vaso-dilator effects, the impression still prevails that it raises blood pressure by peripheral constriction. Our experiments on the rate of blood flow and other associated phenomena indicate that the main action of adrenalin is upon the output of the heart, which is greatly increased, and that no demonstrable peripheral constriction occurs.

*The Rate of Blood Flow as Determined by a New Method* By FRANCIS W. PEABODY and (by invitation) HERMANN L. BIUMGART and OTTO C. YENS, Boston, Mass

The method utilized is as follows. The active deposit of radium is injected at one point and its time of arrival at another point noted. In rabbits, the marginal ear vein and the foot were the two places arbitrarily chosen.

The various devices for detecting the presence of minute quantities of radium depend on the radiations continually emitted from the radium atom. These radiations can penetrate tissues and ordinary materials but can be completely stopped by sufficient thicknesses of lead. The animals are separated from the detecting device by thick blocks of lead except at one point. When the blood carrying the radium active deposit reaches that point, the radiations, instead of being stopped by the lead, will penetrate through the air and be registered by the detector.

For the purpose of detection we have utilized a needle electrode ionization chamber and also a cylindrical ionization chamber. Within the past few weeks we have had promising results using the cloud method of C. T. R. Wilson. On man, the active deposit has been injected into the vein of one arm and detected at the wrist of the other arm. At present we are making further observations on human subjects to determine more accurately the clinical feasibility of this method.

*The Effect of Excluding Pancreatic Juice from the Duodenum on the Motility of the Stomach and Small Intestine* By J. H. PRATT and (by invitation) L. WHITAKER, Boston, Mass

Study was made on dogs in which the pancreas had been completely separated from the duodenum. The passage of food from the stomach through the intestines was determined by the Roentgen ray. The stomach emptied much more rapidly than in health and the passage of food through the upper portion of the small intestine was accelerated.

*The Absorption of Bile Pigment from the Intestine* By M. A. BLANKENHORN, Cleveland, Ohio

A method is presented to study the bile contents of dogs' portal vein blood. Portal vein blood was compared with jugular vein blood as to the content of bile pigment. There is evidence to show that portal vein blood contains more urobilin than does jugular vein blood, and that absorption of urobilin takes place from the intestine.

*An Hypothesis Concerning the Transportation of Water in the Body* By THOMAS E. BUCKMAN and (by invitation) DAN C. DARROW, Boston, Mass

Since Starling demonstrated the magnitude of the osmotic pressure exerted by the colloids of the blood plasma, it has been generally held that the resorption of water on the venous side of the capillary meshwork is due principally to the fall in the hydrostatic pressure of the blood.

Evidence is here presented to show that another mechanism may influence the transfusion of water between blood and tissues. This mechanism is dependent on the increase in the osmotic pressure of the blood plasma which accompanies increasing tensions of carbon dioxide to which the whole blood is exposed, a change in a general way proportional to the increase in the bicarbonate content of the plasma.

The effective pull of the blood in regions of higher carbon dioxide tension would depend, in part, on the difference between the bicarbonate content of arterial and venous plasma. This difference, in turn, would be influenced by (1) the shape of the carbon dioxide dissociation curve of the blood and (2) the differences between the tensions of carbon dioxide and oxygen in the lungs and tissues.

Some data concerning the causes of shape differences in different carbon dioxide dissociation curves are presented and also certain clinical observations which might be accounted for on the basis of the conception here set forth.

*Transfusion of Lymphocytes and Their Rapid Disappearance from the Circulation of Man* By RAPHAEL ISAACS and GEORGE R. MINOT, Boston, Mass.

Whole blood from a case of chronic lymphatic leukemia (white blood cells 89,000 per cubic millimeter, 95 per cent lymphocytes) was transfused into a patient with generalized lympho-sarcoma (white blood cells 6400 per cubic millimeter, 15.5 per cent lymphocytes, hemoglobin, 50 per cent). The lymphocytes in the peripheral circulation of the recipient increased to 42.5 per cent after the transfusion, but dropped to 25 per cent in one-half hour and reached the pretransfusion percentage within two and one-quarter hours.

*Factors of Dehydration Following Pyloric Obstruction*<sup>2</sup> By JAMES L. GAMBLE, M.D., and (by invitation), MUNROE A. MCIVER, Boston, Mass.

Data obtained from rabbits consist of measurements of water, chlorides and fixed base in the gastric contents of controls and after closure of the pylorus. They show, following pyloric obstruction, a secretion of water, chlorides and fixed base into the stomach of from two to three times the estimated initial total plasma content.

A chief point of these findings is the large loss of fixed base. From the point of view of dehydration, the loss of base is the significant factor since it represents an absolute depletion of the body's content of dissolved electrolytes, whereas loss of chloride ion is replaced by increase of bicarbonate ion.

Haden and Orr regard the action of sodium chloride as protective against a toxic substance rather than simply reparative of dehydration. They state that the chloride lowering in the plasma is only in part explained by loss in vomited secretions. They find that it occurs when there is little vomiting and in rabbits which

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<sup>2</sup> A complete report of this work appears in THE JOURNAL of CLINICAL INVESTIGATION, 1925, 1, 531.

cannot vomit. They believe that chloride leaves the plasma in offensive quest of a toxic substance.

Our rabbits did not vomit. They, however, lost into their stomachs several times the total plasma capacity for chloride. That any chloride at all is found in the plasma proves a movement in the direction opposite from that surmised by Haden and Orr.

*The Effect of Iodine by Mouth on the Reaction to Intravenous Injections of Thyroxin*

By CYRUS C. STURGIS and (by invitation) SALVADOR ZUBIRAN, GUY W. WELLS and THEODORE BADGER, Boston, Mass.

The recent observation that the oral administration of iodine results in a striking reduction in the basal metabolism of patients with exophthalmic goiter adds new information to our knowledge which may have important bearing on the physiology and physiological pathology of the thyroid. It is not clear, however, whether the principal mode of action of iodine in producing this remission is by causing some anatomical alteration in the thyroid gland or by exerting some effect on the product secreted by the gland. In an endeavor to gather further information on this subject, rabbits were injected with small doses (1 mgm. on 3 successive days) of thyroxin, and their response determined by recording their pulse rate, body-weight, and oxygen consumption daily for a long period. The same animals were then given Lugol's solution orally for a variable period and again received similar injections of thyroxin. There was no evidence that the iodine had any effect on the action of thyroxin as the animals responded in the typical manner. If exophthalmic goiter is due to pure excess of the thyroid secretion, which some hold, then the action of iodine in producing a remission is not due to its effect on the circulating thyroxin.

*The Static and Kinetic Representations of the Efferent System in the Psychic Sphere*

By J. RAMSAY HUNT, New York, N. Y.

In previous communications the dual nature of the efferent nervous system has been presented and its bearing on the interpretation of motor disorders. According to this conception, movement is subserved by a kinetic, and posture by a static mechanism, which present a parallelism of function and structure at all physiological levels of the efferent nervous system, vegetative and cerebro-spinal.

This paper considers the kinetic and static representations of the efferent system in the purely psychic sphere. Evidence is presented in favor of the view that thinking is a *kinetic* representation, while decision, conclusion and belief are representations of *static* function. Therefore movement and posture are represented in mental processes by thinking and belief respectively. The relation of thinking and belief to certain morbid mental processes is considered.

*The Resistance of Immature Erythrocytes to Heat*<sup>\*</sup> By RAPHAEL ISAACS and GEORGE R. MINOT, Boston, Mass

The red blood corpuscles of normal human blood, when heated to 55°C for one-half hour, undergo a profound and characteristic modification, with the production of fragmented forms, "shadows," microcytes, poikilocytes and a uniform distribution of the hemoglobin throughout the cell. No noteworthy visible changes take place at 45°C to 50°C but at 65°C complete destruction occurs. Immature erythrocytes, reticulocytes, polychromatophilic cells and granule red cells, of both normal and pathological blood are much more resistant when heated to 55°C than mature erythrocytes, with the apparent exception of reticulated megalocytes. The difference between the effect of heat on the red cells of normal and pathological blood is not qualitative, it is quantitative, proportional to the number of immature cells.

There are two kinds of red cells which show no histological evidences of immaturity, and are classed as mature corpuscles. One kind resists the action of heating to 55°C while the other is broken up and altered. The former, represent a majority of the red blood corpuscles in chronic hemolytic jaundice so that the bulk of the red cells of this condition remain conspicuously intact after heating. They are probably the younger of the mature cells.

*A Report of Fifty Patients suffering from Graves' Disease and under Observation for Four Years* By LEO KESSEL and (by invitation) H. T. HYMAN, New York, N. Y.

A report of the further progress of fifty patients who suffered from Graves' Disease and who are now at the end of the fourth year of observation, who have received no specific therapy, whose progress has been estimated by subjective symptoms, by objective methods particularly pulse rate and basal metabolism, exophthalmometer readings and weight, and by a standard as to their economic and social restitution. This report is to serve as a control to a series later to be reported, in which the same regime was followed except that a sub-total thyroidectomy was done on series I and in series II Lugol's solution was administered before thyroidectomy was done.

It is hoped that at the completion of the observations of these series a definite therapeutic policy can be established for the management of patients suffering from Graves' disease.

*Studies of the Vascular Features of Polycythemia Vera* By GEORGE E. BROWN (by invitation) and HERBERT Z. GIFFIN, Rochester, Minn.

The basis of the paper is the study of fourteen cases. An increased viscosity was present in all. The highest reading was 11.2 (normal 1.45). With a

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<sup>\*</sup>A complete report of this work appeared in THE JOURNAL OF CLINICAL INVESTIGATION, 1925, 1, 425



normal red cell count the viscosity was frequently increased. The total circulating blood volume was increased in all cases, the average being 10,300 cc or 166 cc for each kilogram. Cell hematocrit values varied from 58 to 70 and averaged 62. In twelve cases in the nail fold the venous limb of capillaries and the collecting venules were markedly engorged, and in four cases the arterial limb was engorged. All available capillaries were utilized. The capillaries reached their normal size when the total circulating blood volume had decreased to 106 cc. for each kilogram. Engorgement of the retinal veins disappeared at the same volume. Cardiac enlargement and cardiac lesions were noticeably absent. The liver was only slightly enlarged. The size of the spleen bore a direct relationship to the degree of total circulating blood volume. The engorgement of the vessels and the increase in viscosity had no effect on the elevation of arterial tension. The venous pressures were slightly higher than normal, the capillary pressures were normal. Renal function was only slightly impaired. Heat production in the foot showed abnormally marked fluctuations. In polycythemia, the capillaries and venules have a storage function, this in association with retardation of flow affects fundamentally the mechanism of heat dissipation.

*Energy Expenditure during Mechanical Work in Obese, Normal, and Thin People*

By SOLOMON STROUSE and (by invitation) C. C. WANG and ZELMA OWEN, Chicago, Ill.

Growing out of the investigations of the energy metabolism of obesity a comparison of the energy expended for mechanical work among obese, normal, and thin subjects was undertaken. Tissot respiration apparatus was employed as in our previous work. The energy expended for mechanical work was measured by a bicycle ergometer.

Thirteen obese, ten normal, and six thin people served as subjects. Normals were those whose weights varied between plus ten and minus ten per cent from their standard weights.

Results are expressed in terms of mechanical efficiency. Figures show that the obese spend more energy on a given piece of work than the normal, and the thin people are more efficient than either of the other groups. The respiratory quotients are invariably increased during exercise and the increase is directly proportional to the mechanical efficiency of the three groups.

*The Reactivation of Inactivated Insulin in Vitro and in Vivo* By ALBERT A. EPSTEIN, New York, N. Y.

Previous studies have established that trypsin can inactivate insulin in vitro and in vivo, and that this function in vivo may play an important rôle in the causation of diabetes.

Insulin may be dissociated from trypsin in vitro by shifting the hydrogen ion concentration of the substances in solution to the acid side of 4.6. It became necessary to ascertain whether dissociation could be effected in vivo. Obviously

the hydrogen ion concentration of the body fluids could not be altered to the point necessary for the dissociation of insulin. Hence other means were tried and these results obtained.

1 Upon addition, *in vitro*, to inactivated insulin (trypsin) of such substances as pepsin, safranin, and cryogenin (m-benzaminosemicarbazide) liberation of insulin takes place, as evidenced by results obtained from injection of these mixtures into suitable test animals.

2 These agents (pepsin, safranin and cryogenin) can dissociate insulin from trypsin *in vivo*. Injection of suitable amounts of these substances (subcutaneously or intravenously) just prior to parenteral administration of inactivated insulin causes its liberation and the production of its physiological effects.

Therefore if diabetes is the result of insulin deficiency caused by inactivation of insulin by trypsin, then the possibility of reactivating insulin *in vivo* may be of practical therapeutic importance.

*Absorption Curve of Spinal Fluid* By A. T. SHOHL and (by invitation), S. KARELITS, New Haven, Conn.

A study is being made of the acid-base equilibrium of spinal fluid, to apply to physiological and clinical problems. The absorption curve has been studied by saturation with the Van Slyke et al. technique by use of a new type of tonometer which permits both saturation and colorimetric pH determination and sampling for CO<sub>2</sub> content without transfer or exposure to air. By this method, values for pK<sub>1</sub> are obtained which permit calculation of the tension of CO<sub>2</sub> in spinal fluid without requiring absorption curves.

*The Influence of Temperature on Acid Base Equilibrium in the Blood* By J. H. AUSTIN and (by invitation) G. E. CULLEN and H. W. ROBINSON, Philadelphia, Pa.

In the study of blood acid base equilibrium under conditions of varying temperature there are four variables of which the temperature coefficient must be taken into account.

1 The solubility of CO<sub>2</sub>.

2 The value of pK' of the Henderson-Hasselbalch equation. Cullen, Keeler and Robinson found

$$\frac{\Delta pK'}{\Delta t^{\circ}C} = -0.005$$

3 Change in the location of the CO<sub>2</sub> absorption curve. This is best stated as the change in pH with temperature at constant [BHCO<sub>3</sub>]. Stadie and Martin found for whole human blood

$$\frac{\Delta pH}{\Delta t^{\circ}C_{[BHCO_3] \text{ constant}}} = -0.022$$

normal red cell count the viscosity was frequently increased. The total circulating blood volume was increased in all cases, the average being 10,300 cc or 166 cc for each kilogram. Cell hematocrit values varied from 58 to 70 and averaged 62. In twelve cases in the nail fold the venous limb of capillaries and the collecting venules were markedly engorged, and in four cases the arterial limb was engorged. All available capillaries were utilized. The capillaries reached their normal size when the total circulating blood volume had decreased to 106 cc. for each kilogram. Engorgement of the retinal veins disappeared at the same volume. Cardiac enlargement and cardiac lesions were noticeably absent. The liver was only slightly enlarged. The size of the spleen bore a direct relationship to the degree of total circulating blood volume. The engorgement of the vessels and the increase in viscosity had no effect on the elevation of arterial tension. The venous pressures were slightly higher than normal, the capillary pressures were normal. Renal function was only slightly impaired. Heat production in the foot showed abnormally marked fluctuations. In polycythemia, the capillaries and venules have a storage function, this in association with retardation of flow affects fundamentally the mechanism of heat dissipation.

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*The Influence of Various Factors upon the Length of Systole in Man as Measured by the Electrocardiogram* By HOWARD B SPRAGUE (by invitation) and PAUL D WHITE, Boston, Mass

Our investigations have verified the well known fact that the most obvious influences determining the length of systole are the factors which control the rate of the heart. These are probably various influences concerned in the nervous mechanism of the heart beat. The duration of systole in a normal control series of adult males and females and children has varied from 0.4404 second at a pulse rate of 53, to 0.2637 second at a rate of 120. In addition to this normal relationship between heart rate and systolic length the influence of other factors, such as cardiac failure, blood pressure, heart size, drug therapy, exercise, and hyperthyroidism are being studied.

*A Preliminary Note on the Blood and Cerebrospinal Fluid Chlorides in Chronic Nephritis and Uremia* By JOHN B YOUNG (by invitation) and FRANK N WILSON, Ann Arbor, Mich

Studies of the influence of various ions in convulsive states, as tetany, and the investigations by Weed and Wegeforth and by Collip on the effect of intradural injections of electrolytes, suggest that an increase in cerebrospinal fluid chlorides may play a rôle in the production of convulsive uremia.

To investigate this possibility the concentration of chlorides in the blood and cerebrospinal fluid of patients with chronic nephritis, with and without uremia, was determined.

Twenty-one patients were studied. Seven developed convulsive uremia. Of the seven, four had 800 to 840 mgm. of chlorides per 100 cc. of cerebrospinal fluid, and one 770 mgm. Two had normal amounts, but agonal changes may have influenced the results. Fourteen patients without uremia gave normal findings, except one who had 792 mgs.

Simultaneous blood chloride determinations showed increased concentrations in patients with increased chlorides in the cerebrospinal fluid, but increased blood chloride concentrations occurred independently of increased cerebrospinal fluid chlorides or uremia. No constant relation was established between increased spinal fluid chlorides and such factors as blood non-protein nitrogen, blood pressure edema, etc.

Eleven patients without nephritis (normal, syphilis, cardiac failure, etc.) gave low or normal figures for the chlorides of the cerebrospinal fluid.

*Intracardiac Fistulae* By C S BECK (by invitation), E C CUTLER and (by invitation) EMILE HOLMAN, Cleveland, Ohio

Experiments were performed in which fistulae between the heart chambers were established in an attempt to study the circulation under the conditions imposed in certain forms of congenital heart disease. It appeared to us that the dilatation of the heart chambers and the muscular hypertrophy found repeatedly

We find this value to be  $-0.02 \pm 0.003$  for whole blood, true serum and separated serum of both dog and sheep

4 The change in the neutral point, i e,  $1/2$  pK water Between  $10^{\circ}$  and  $45^{\circ}\text{C}$  this is about

$$\frac{\Delta (\frac{1}{2} \text{ pK water})}{\Delta t^{\circ}\text{C}} = -0.014$$

*The Coating of Bacteria by Agglutinin in Specific Bacterial Agglutination* By GERALD S SHIBIEY (by invitation) and A R DOCHIEZ, New York, N Y

The specific charge reducing effect of agglutinating sera observed in work with paratyphoid bacilli and pneumococci has been determined for ten additional organisms Where the cataphoretic charge is high without serum, it is specifically reduced, where low, it is raised In all cases, no matter what the initial charge (range, 40 to 5 mv), high concentrations of sera bring the charge to a *common potential level* (8 to 14 mv) Loeb has shown that protein coated collodion particles take the cataphoretic charge of these proteins Sensitized pneumococci compared with pneumococcus antiserum euglobulin particles (agglutinin) show identical charges Probably, therefore, the first step in specific agglutination is selective coating of bacteria by specific agglutinins, and charge changes observed are the result of this Loeb has shown that collodion particles coated with crystallin egg albumin behave, as to stability in suspension, as if the albumin film were denatured Bacteria treated with agglutinating sera (coated) act also like denatured proteins It is probable that the specific globulins of agglutinating sera that coat bacteria are similarly denatured and that the resulting flocculation is due to this "denaturation" Preliminary experiments with globulins and collodion particles bear out this explanation of the mechanism of specific bacterial agglutination

*Interruption of Complete Heart Block by Sequential Beats in Early Diastole Exemplifying Recovery Phase of Cardiac Muscle* By CHARLES C WOLFERTH, Philadelphia, Pa

A patient in whom prolonged conduction periods were observed for two years finally developed complete dissociation, although sequential type of beating could be temporarily restored by atropin Several times, tracings were obtained showing interruption of complete dissociation by response to auricular beats falling within a certain range of early diastole This temporary recovery of ability to transmit impulses has been reported by Lewis and Master who attribute it to "supernormal recovery phase" described by Adrian and Lucas The number of clinical cases now described (three) undoubtedly demonstrates a tendency during heart block toward a recovery phase of junctional tissues in early diastole That this recovery phase is of the nature of the response described by Adrian and Lucas involves assumptions that can not be established at present

A girl aged seven years, routinely rayed prior to tonsillectomy, showed thymus hypertrophy. After three radiations, weight increased about 7 pounds within a month.

*The Mechanism of Death from Quinidine and Methods of Resuscitation. An Experimental Study.*<sup>4</sup> By BURGESS GORDON and MARCEL MATTON (by invitation) and S. A. LEVINE, Boston, Mass.

Unexpected and unexplained deaths have occasionally occurred during the clinical administration of quinidine in treatment of certain heart conditions. One fatality we observed showed a peculiar difficulty in breathing and presented a picture of a more general toxic state than is ordinarily seen with heart failure. Death was gradual and autopsy showed no evidence of emboli.

With the above experience in mind, the method of death in quinidine was studied in cats. It was found that although definite heart intoxication took place, a most important factor that has been hitherto neglected was also involved, i.e., the respiratory mechanism. It was a constant finding that the respiration failed before death and that this failure of respiration could not be entirely accounted for by a lowered state of the circulation. It was also found that when respiration had ceased, following such doses of quinidine as many previous experiments had indicated to be fatal, if artificial respiration were instituted, the animal could be saved and complete recovery take place. The administration of caffeine had a similar action in resuscitation, but was not as dependable. The best and most speedy results were obtained when both artificial respiration and caffeine were employed.

*The Storage of Inorganic and Food Iron in the Liver, Spleen and Bone Marrow.* By CHARLES SPENCER WILLIAMSON, Chicago, Ill.

The question as to the storage of iron has been one that has been answered both pro and con. The question is intimately connected with the formation of hemoglobin. In a series of experiments, which have been carried on for about two years, the results of which are being reported at another meeting, the writer has shown that inorganic, medicinal iron may be stored up in large quantities in these organs.

The present research has been undertaken to see whether true organic iron, that is food iron, is stored up in the same way, and if so, whether such storage adds to the available reserve supply of hemoglobin. Along with this, the behavior of the blood hemoglobin has been studied with methods of precision, spectrophotometrically.

This question is of considerable importance in that it points out a possibility of

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<sup>4</sup> A complete report of this work appears in *THE JOURNAL OF CLINICAL INVESTIGATION*, 1925, 1, 497.

at autopsy in cases of patent interauricular and interventricular septa were unexplained by previous conceptions. It was our feeling that the interpretation of peripheral fistulae, as demonstrated experimentally and clinically by one of us might well be applicable to these congenital conditions, i e, the changes might be due to increased blood flow through that portion of the system into which the larger stream of blood is diverted by the defect.

Interventricular fistulae were established by the use of knives and the modified cardiovalvulotome, used by us in the establishment of valvular insufficiencies. Under these conditions studies of blood pressure, blood volume, changes in the heart size as demonstrated by the X-ray, and subsequent examinations were conducted. These studies demonstrated that the capacity of the chambers of the heart and the alterations in their musculature seem to be determined by the minute volume flow through them.

*Metabolism Studies in Exophthalmic Goiter Complicated by Diabetes* By WALTER M. BOOTHBY and RUSSELL M. WILDER, Rochester, Minn.

Approximately complete metabolism data on two patients having exophthalmic goiter complicated by diabetes were presented. These studies, supported by several other cases less completely investigated, showed that the exophthalmic goiter syndrome materially reduces the ability of a diabetic patient to utilize carbohydrate, decreases the efficiency of a unit of insulin and increases the danger of a sudden onset of diabetic coma. The marked improvement in the ability of the patient to utilize carbohydrate, as the exophthalmic goiter syndrome is controlled by iodine (Lugol's solution), was clearly brought out. The clinical importance of recognizing the exophthalmic goiter complication in cases of diabetes was emphasized and it was pointed out that this complication should be suspected as the possible significant factor in those diabetic patients who are not readily controlled by ordinary measures.

*Thymus Enlargement* By H. GRAY, Santa Barbara, Cal.

In any child whose growth is retarded, thymus enlargement merits a thought. Three suggestive cases follow.

A boy aged six years was coughing. X-ray showed a broad shadow in the second interspace. Diagnosis of tuberculous nodes was rejected in favor of the thymus. After the photograph alone, the cough improved and was completely relieved after three radiations. Re-ray showed diminution of the shadow. During the ensuing year growth was rapid in height 45 per cent and in weight 131 per cent more than the average yearly increase for his age.

A second boy, aged nine years, was backward compared with his brother a year younger, height was 10 per cent sub-normal, teeth were notched and occlusion poor, right testicle intra-abdominal. Thymus enlargement was suspected, confirmed by x-ray. Radiated six times and being watched for acceleration in development.

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increasing the available reserve supply of hemoglobin, and especially so since there is no considerable reserve supply of iron in the body, almost 90 per cent of it being found in the blood

*Mitral Stenosis after the Fifth Decade of Life* By ERNST P BOAS and (by invitation) DAVID PERLA, New York, N Y

Mitral stenosis is not uncommon in the sixth and seventh decades of life Forty-six of 183 consecutive cases of mitral stenosis studied at Montefiore Hospital were over 50 years of age In most instances it is the end result of a rheumatic infection in childhood or early adult life The mitral narrowing is not extreme and the lesion is nonprogressive, or very slowly progressive, so that significant symptoms do not develop for many years Women with such a condition may apparently undergo many pregnancies without distress and without injury to the heart

In a certain number of cases of mitral stenosis in elderly persons, the valvular lesion cannot be interpreted as the end result of an ancient endocarditis but must be regarded as a primary atherosclerosis of the mitral valve and particularly auriculoventricular ring The characteristic pathological finding is a widespread calcification of the mitral ring, encroaching on the lumen of the auriculoventricular opening with thickening and at times calcification and fusion of the valve cusps

*Cardiographic Differentiation of a Sub-group of Intra-ventricular Block with Observations on the Prognosis* By B S OPPENHEIMER, M A ROTHSCHILD, and (by invitation) HUBERT MANN, New York, N Y

In a series of ten patients a type of electrocardiogram was observed with such characteristics that theoretical considerations led us to believe the prognosis would be less serious than in the usual type of arborization block The electrocardiograms of this group show marked widening and notching of the Q R S complex, but they differ from the usual arborization block in that the changes are confined almost entirely to the terminal portion of the second limb of the R wave The Q R S portion of the electrocardiograms bear a striking resemblance to one another The voltage, T wave, and P-R interval present no noteworthy abnormalities The teleor-entgenograms show moderate widening of the aortic arch

In a previous series of intraventricular block, the mortality was 52.1 per cent and the average duration of life after the initial electrocardiogram was only 8 months In contrast to this poor prognosis, these ten patients with a solitary exception, showed no downward progress either clinically or electrocardiographically The patients have been followed for from 1 to 9 years, an average of  $3\frac{1}{2}$  years The most recent case, and the only syphilitic of the series, died of a lobar pneumonia The remaining nine are ambulatory and follow their customary occupations

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